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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

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Volume XLIII, No. 7

July, 1957

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO.	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

PHYSIOLOGY

ELECTROPHYSIOLOGIC CHARACTERISTICS OF INTEROCEPTIVE REFLEX ARC

COMMUNICATION I. CHARACTERISTICS OF REFLEXES ARISING ON STIMULATION OF BLADDER MECHANORECEPTORS

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(Received May 19, 1956. Submitted by Active Member AMS USSR Professor V. N. Chernigovsky)

Reflexes arising on stimulation of various interoceptive areas have been well studied but there are still no complete data on processes occurring in various links of the interoceptive reflex arc during the appearance and development of an interoceptive reflex.

In order to study the processes which take place in the interoceptive reflex arc the reflexes associated with stimulation of the bladder mechanoreceptors were selected in the present work. Physiologic investigations and clinical data provide evidence of complex reflex connections between the organs of the excretory system in the first place. Thus, Barrington [7, 8] described a series of reflexes appearing in cats during the act of micturition: reflex contraction of the bladder associated with the passage of fluid through the urethra; reflex relaxation of the urethra during the passage of fluid through it; reflex relaxation of the urethra accompanying distention of the bladder musculature.

Other authors studied action potentials in afferent and efferent fibers of the bladder [10, 12].

It was shown that depending on the functional state of the central nervous system micturition can occur at different degrees of filling of the bladder [2, 3, 9, 11].

N. A. Adamovich [1], who studied afferent impulses in the branches of the pelvic nerve which innervates the bladder noted that the intensiveness of efferent activity in the pelvic nerve depended not only on the intensiveness of afferent activity from the bladder but also on the state of the central nervous system. When the excitability of the centers had been artificially increased by strychnine the efferent activity in the pelvic nerve was considerably enhanced and could even be observed when the bladder was empty.

Stimulation of the bladder mechanoreceptors leads not only to reflex contraction of its musculature and to reflex changes in the tonus of the urethra but also produces reflex changes in other systems of the organism. Changes in blood pressure, respiration, activity of smooth and skeletal muscle etc. may occur during stimulation of the bladder mechanoreceptors. Some time ago V. N. Chernigovsky [4, 5] had suggested that two types of interoceptive reflexes be recognized – "direct" and "conjugated". The former are reflexes directly connected with a given physiologic system and discharged within its limits, mediating a definite group of reflexes "serving" the given system. The latter, also arising from the given interoceptive area, are discharged outside the given

physiologic system and involve in the reaction physiologic systems which are not directly connected with the first one in a functional sense.

Subsequently V. M. Khayutin [6] established that "direct" reflexes (from the bladder mechanoreceptors to urethral tonus) had a 2-7 times lower threshold than the "conjugated" reflexes (from the bladder mechanoreceptors to blood pressure and respiration) and were much less subject to adaptation on prolonged stimulation of the bladder mechanoreceptors. Reflexes described by Barrington should be regarded as "direct".

In the present communication data are presented concerning the electrophysiologic characteristics of "direct" reflexes primarily (the electrophysiologic analysis of "conjugated" reflexes will form the substance of further communications).

METHODS

Experiments were performed on cats. Intravenous urethane anesthesia was used. In order to stimulate the bladder mechanoreceptors it was filled with physiologic solution or with air. A cannula was introduced into the bladder through the urethra; the cannula was connected by a three-way tap with a system of vessels used for inflation and with a mercury manometer which registered pressure within the bladder. The tonus and contractions of the urethra were recorded by the method described by V. M. Khayutin [6]. In these experiments carotid artery blood pressure was also recorded (mercury manometer).

Simultaneously with the recording of blood pressure and urethral tonus a record was taken of the electric potentials in the peripheral end of a branch of the pelvic nerve leading to the bladder (afferent impulses from bladder receptors) and in the central end of the pudendal nerve (efferent impulses to the urethra).

Nerve action potentials were led off by means of platinum electrodes with an interelectrode distance of 4-5 mm; a two-channel cathode oscillograph "OB-2" and amplifier were used. The frequency characteristic of the amplifier was linear within the range of 10-500 cps.

RESULTS

Recording of action potentials from the peripheral end of the pelvic nerve (afferent impulses from the bladder) when the bladder was empty showed that electric activity was absent from the nerve, which is in accordance with the data of other authors. At the same time continuous impulses were observed in the central end of the pudendal nerve; these impulses increased somewhat when physiologic solution was allowed to flow through the urethra. Introduction of from 2 to 10 cc of air (different quantities in different experiments) into the bladder gave rise to considerable increase of afferent impulses in the pelvic nerve. The central reaction in response to this stimulation was an increase in the frequency and amplitude of efferent impulses in the pudendal nerve. The latent period of the change in efferent impulses following the beginning of stimulation of the bladder mechanoreceptors was from 2 to 7 seconds. There was simultaneous increase in the tonus and contraction of the urethra (Fig. 1).

Thus the first reflex which appears on weak stimulation of the bladder mechanoreceptors is an increase in the tonus of the urethra.

There are no changes in blood pressure.

Increasing the intensity of stimulation of the bladder mechanoreceptors (introduction of 10-20 cc of air into the bladder) caused strong afferent activity in the pelvic nerve.

In response to increased stimulation there was lowering of efferent activity in the pudendal nerve.

On further increasing bladder stimulation efferent impulses in the pudendal nerve ceased altogether. The latent period for change in efferent activity remained the same. During this time decrease of urethral tonus, i.e., its relaxation, was observed (Fig. 2). No changes in blood pressure occurred with this strength of stimulation.

Only on still further increase in the strength of stimulation did a rise in blood pressure take place together with complete depression of efferent impulses and relaxation of the urethra, i.e., the reflex influences spread not only to the system directly related to the performance of the given function, but also involved a system further removed in a functional sense.

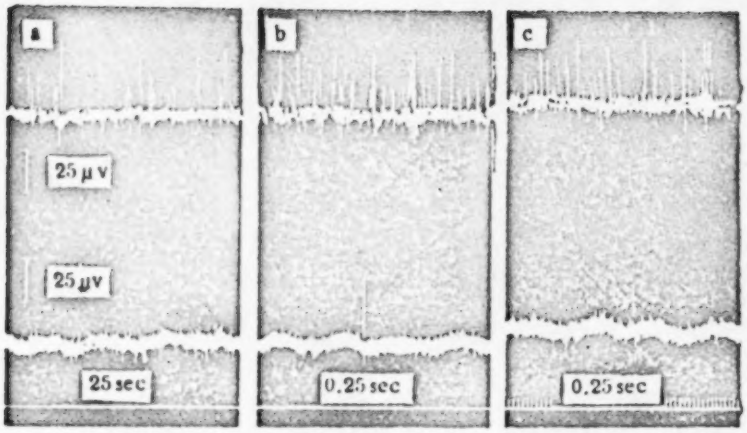

Fig. 1. Reflex changes in urethral tonus and afferent and efferent impulses on weak stimulation of the bladder mechanoreceptors.
a) Initial efferent impulses in the pudendal nerve with a rhythm of 60 per second. Afferent impulses in the pelvic nerve are absent (before stimulation); b) 9 cc of air introduced into the bladder; efferent impulses in the pudendal nerve with a rhythm of 100 per second. Afferent impulses in the pelvic nerve represented on the oscillogram by several impulses; c) the same 5 minutes after withdrawal of air from the bladder; efferent impulses in the pudendal nerve with a rhythm of 70 per second; afferent impulses in the pelvic nerve absent. Records from above down: action potentials of the central end of the pudendal nerve; action potentials of the central end of the pelvic nerve; time marker (0.25 seconds).

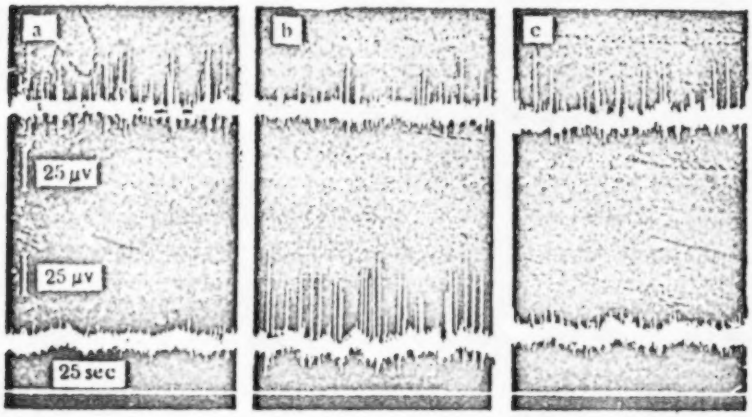

Fig. 2. Reflex changes of urethral tonus and of afferent and efferent impulses on stronger stimulation of the bladder mechanoreceptors.
a) Initial efferent impulses in the pudendal nerve with a rhythm of 70 per second. Afferent impulses in the pelvic nerve absent (before stimulation); b) 11 cc air introduced into the bladder; efferent impulses in the pudendal nerve at 32 per second; appearance of considerable afferent activity in the pelvic nerve; c) the same 5 minutes after withdrawal of air from the bladder; efferent impulses in the pudendal nerve with a rhythm of 55 per second; afferent impulses in the pelvic nerve absent. Records the same as in Fig. 1.

In order to trace the part played in natural micturition by those reflex relations between the bladder and

the urethra which were observed in the present work with various degrees of filling of the bladder, special experiments were performed in which the abdominal cavity was opened with great care. In those cases in which the bladder proved to be filled with urine afferent impulses were recorded from the pelvic nerve and efferent impulses from the pudendal nerve.

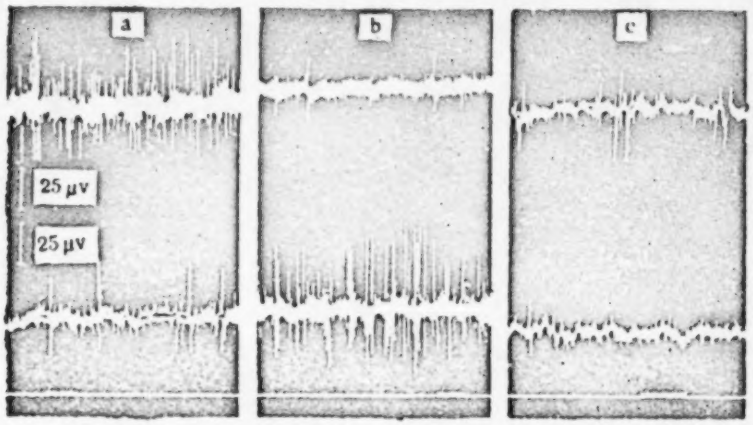

Fig. 3. Reflex effects from the bladder on urethral tonus; efferent and afferent impulses during natural micturition.
a) Bladder is filled with urine, but is relaxed; strong efferent activity is seen in the pudendal nerve and weak afferent activity in the pelvic nerve;
b) contraction of the bladder during micturition; sharp lowering of efferent activity in the pudendal nerve; intensive afferent activity in the pelvic nerve;
c) the same after emptying of the bladder; efferent impulses in the pudendal nerve present but considerably weaker than with full bladder; afferent impulses in the pelvic nerve practically absent; records the same as in Fig. 1.

Figure 3, a shows that if the bladder is filled but relaxed low frequency afferent activity occurs in the pelvic nerve. At the same time strong efferent impulses to the urethral musculature is observed in the pudendal nerve and it is evidently these impulses that maintain the urethra in the contracted state, thus preventing the escape of urine from the bladder. Further filling of the bladder is thus possible.

However, when the bladder reaches certain dimensions its contraction begins. As can be seen from Fig. 3, b, contraction of the bladder gives rise to considerable afferent activity in the pelvic nerve under the influence of which a sharp decrease in the efferent activity with respect to urethral musculature and its relaxation occur. This mechanism ensures emptying of the bladder. Following evacuation of the bladder (Fig. 3, c) efferent impulses to urethral musculature are decreased considerably as compared to impulses seen when the bladder is full (Fig. 3, a).

At this moment, as shown by V. M. Khayutin [6], if the outflow of urine is blocked, a rise of blood pressure takes place.

DISCUSSION

The results obtained show that two opposite reactions of the urethra can be obtained on simultaneous recording of afferent impulses from the bladder and efferent impulses to the urethral musculature, as well as of urethral tonus and blood pressure, depending on the strength of stimulation of the bladder mechanoreceptors.

Weak stimulation of bladder mechanoreceptors gives rise to insignificant afferent impulses in the pelvic nerve. Reaching the centers, they elicit a state of excitation if the state of the centers is judged by the efferent impulses in the pudendal nerve, since the latter are increased. It must be supposed that this reflex underlies the mechanism of bladder filling up to a definite volume. This volume varies not only in different animals but also in one and the same animal depending on the functional state of the central nervous system [2, 3, 9, 11].

Increased stimulation of the bladder mechanoreceptors leads to the appearance of intensive afferent activity in the pelvic nerve which also finds its way to the centers. In this case this activity evidently leads to the development of inhibition, since efferent impulses in the pudendal nerve become markedly weaker or cease altogether. Dilatation of the urethra follows cessation of efferent activity in the pudendal nerve supplying the urethral musculature. It is this reflex which makes reflex emptying of the bladder possible. The role of this reflex in micturition has been studied previously [6, 7, 8, 10].

Both these reflexes fit into the framework of "direct" reflexes. Further increase in the strength of stimulation of the bladder mechanoreceptors in the present experiments gave rise to changes in blood pressure in addition to complete inhibition of efferent impulses and relaxation of urethral tonus.

Consequently, both these "direct" reflexes are at the basis of micturition. Until the intensiveness of impulses in the afferent fibers reaches a definite strength, efferent impulses maintain the urethra in a state of active contraction. Only on further increasing the strength of stimulation does inhibition of efferent impulses occur. This inhibition, as already suggested, occurs as a consequence of inhibition of relevant centers.

On still further increase of stimulation inhibition in the "centers" of the "direct" reflex becomes still more profound and a "conjugated" reflex appears – a rise in blood pressure, i.e., excitation spreads to other centers.

These data confirm the presence of lower thresholds for "direct" reflexes as established by V. M. Khayutin [6], as well as the general thesis concerning "direct" and "conjugated" reflexes proposed by V. N. Chernigovsky [4, 5].

SUMMARY

Afferent impulsation in the peripheral end of a pelvic nerve branch and efferent impulsation to the urethra in the central end of the pudendal nerve have been recorded in the course of short experiments on cats. Pressure in the urinary bladder, blood pressure and contractions of the urethra have been simultaneously registered. Slight distention of the urinary bladder to 10-20 mm Hg by inflation of 2-10 cc of air caused insignificant afferent impulsation in the pelvic nerve, increased the frequency and amplitude of efferent impulsation in the pudendal nerve, and a rise of the tonus and contractivity of the urethra. Distention of the urinary bladder to 20-30 mm Hg by inflation of 10-20 cc of air intensified afferent impulsation in the pelvic nerve, lowered efferent impulsation in the pudendal nerve down to complete cessation and relaxation of the urethra. Excitation of intermediate degree led to rhythmic bursts and depression, likewise to alternating contraction and relaxation of the urethra. The same three phases have been noted during natural urination.

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NERVOUS REGULATION OF THE RHYTHM OF CARDIAC CONTRACTIONS IN FISH

COMMUNICATION I. THE EFFECT OF STIMULATION AND SECTION OF THE CARDIAC BRANCHES OF THE VAGUS ON CARDIAC RHYTHM

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(Received November 23, 1956. Submitted by Active Member AMS USSR Professor V. N. Chernigovsky)

The concept of a purely inhibitory function of parasympathetic nerves in the vertebrates which arose in the middle of the last century has been under review in recent years. M. G. Udelnov, in a series of communications [3, 4, 5], has shown that both inhibitory and stimulating influences on the heart are mediated by the vagus. It has been established that both regulatory effects are mediated by similar parasympathetic elements. Inhibition or stimulation of cardiac activity is determined, as demonstrated by Udelnov's experiments, by the number of parasympathetic nervous elements whose impulses impinge simultaneously on the heart. When a relatively small number of vagal nervous elements are stimulated cardiac activity is enhanced, when a larger number of such elements is stimulated inhibition takes place. These data were obtained on animals (predominantly amphibia) which had both parasympathetic and sympathetic innervation of the heart, but the results remained unchanged if the experimental animals were subjected to preliminary sympathetic denervation of the heart. It appeared to be important to discover whether the vagi in animals lacking sympathetic innervation of the heart had the power to stimulate automatic cardiac activity.

In the cyclostomata the sympathetic trunk is absent [15], in myxine and in minnow larvae there is no innervation of the heart at all [11]. Fish have a well-defined sympathetic chain, starting from the tail segments and reaching the level of the III-IV thoracic vertebrae [15]. Sympathetic nerves innervate many viscera in many cases although, according to Stannius [15], who investigated over one hundred species of various fish, no nerve fibers of sympathetic origin approach the heart. These data were repeatedly confirmed in more recent studies by morphologists and physiologists [9, 10 et al].

V. A. Shidlovsky [6], who carried out simultaneous recording of venous sinus, atrial and ventricular contractions in some bony fish, observed that on stimulation of the sympathetic "border" trunk a small positive inotropic effect on the venous sinus was seen; however, neither the rhythm of contractions of this structure nor any indicators of the activity of the other parts of the heart showed any changes in the fish examined by him. The venous sinus in these fish is a subservient section of the heart, since the leading cardiac bundle in their case is in the atrial auricle. If this is considered together with the absence of changes in the acitivity of the atria and ventricles in this case, then it must be recognized that this author, too, confirmed the absence of functionally active sympathetic innervation of the heart in fish.

A number of investigators [1, 2, 8, 9, 10, 12, 13, 14, 15] observed cardiac arrest on direct stimulation of the vagi in fish. According to these authors no stimulation of the heart beat was seen either during stimulation itself or subsequently, following arrest evoked by vagal stimulation. This permitted Skramlik [12, 13, 14] to

formulate his theory of unilateral regulation of the heart in fish, mediated by reflex changes in the inhibitory tonus of the vagi. According to his concepts, acceleration of the heart in fish does not occur as the result of active impulses from the vagi but from weakening of impulses from these specifically inhibitory nerves.

It can be concluded from the data available in the literature that fish are a very convenient object for the experimental solution of the problem whether active vagal influence on the heart devoid of sympathetic innervation can cause acceleration of the heart rate.

METHODS

The work was carried out at the Shcherbakovsky water reservoir in July-September 1949 and January-February 1950. Sixty short experiments on fish were performed: mainly on pike, also on tench, perch, carp, grounding bream and nalim *. No significant differences in the innervation of the heart were found in these fish.

The fish were taken from the water and fixed in a frame, on the back. The posterior part of the gill cover was removed, the membrane joining the shoulder girdle with the last gill arch was severed, the superficially lying nerve, a branch of the vagus in the fish, was dissected and traced to the root of the vagus. The other branches of the vagus were then dissected: 4 gill nerves, upper and lower pharyngeal nerves and the visceral branch. The cardiac branches (usually two on each side) may come off the visceral, pharyngeal or gill branches of the vagus; they enter the cardiac venous sinus with the ducts of Cuvier.

The abdominal wall was transected by a transverse incision at the level of the shoulder girdle; a second incision in the midline was made perpendicularly to the first and towards the head. The abdominal wall muscles overlying the heart and the bones of the shoulder girdle were removed. The heart was freed from the pericardium, a serrefine was applied to the apex and connected to an Engelman lever. Cardiac contractions were recorded on a smoked kymograph.

The distal end of one of the cardiac nerves was placed on stimulating electrodes connected through a switch to an induction apparatus.

RESULTS

On stimulation of the peripheral ends of the cardiac nerves of fish with induction currents of different strength various changes in cardiac activity were observed.

In some cases more or less prolonged cardiac arrest was seen (Fig. 1) which was usually replaced, still on the background of stimulation of the cardiac nerve, by cardiac contractions, first infrequent, then increasingly frequent (Fig. 1, b, c) – the so-called "phenomenon of cardiac escape from vagal influence." On cessation of stimulation the slowed rhythm of cardiac contractions sometimes returned immediately to normal (Fig. 1, a), but usually this was preceded by considerable acceleration of the heart rate and only after that was the initial rhythm of cardiac activity regained (Fig. 1, b, c). This is constantly observed in experiments on animals which possess both vagal and sympathetic innervation of the heart. It is explained as a result of the entry into the reaction of sympathetic cardiac nerves and is called "sympathetic after-effect." Neither this name nor the explanation cited above can be applied in the given case, since there is no sympathetic innervation of the heart in fish.

In other cases stimulation of the cardiac branches of the vagi caused only more or less pronounced slowing of the heart rate (Fig. 2).

Finally, in a number of cases stimulation of the cardiac branches of the vagi led to acceleration of the heart rate (Fig. 3). Since acceleration of the heart rate elicited in animals lacking sympathetic innervation of the heart was in these experiments determined by stimulation of the peripheral ends of the cardiac branches of the vagus, explanation of this phenomenon by Skramlik's theory cannot be accepted.

This fact poses the question as to whether inhibitory tonic action of the vagus on the heart exists in fish. Skramlik's concepts were based on the results of his experiments with section of the vagi in fish which caused acceleration of the heart rate.

* A bony fish native to the USSR.

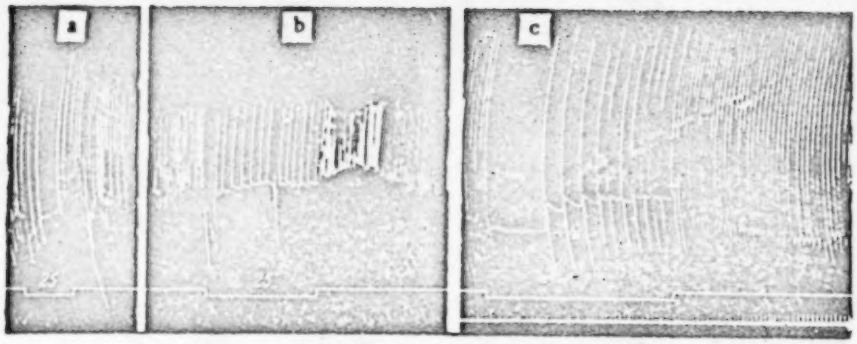

Fig. 1. Cardiac arrest (fish) in response to stimulation of the cardiac branches of the vagus. After cessation of stimulation the heart rate either immediately returns to the initial (a), or exceeds it slightly (b) or very markedly (c), then returning to the initial value. Records from above down: cardiac mechanogram, stimulus mark. Figures indicate distance between the induction coils during given stimulation; time marker.

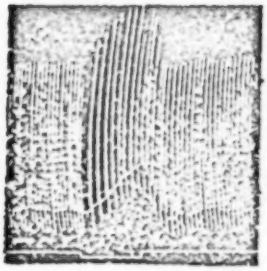

Fig. 2. Slowing of the heart rate (fish) in response to stimulation of the cardiac branch of the vagus. Records the same as in Fig. 1.

Fig. 3. Sharp increase of heart rate during stimulation of the cardiac branch of the vagus. Records the same as in Fig. 1.

In a special series of experiments bilateral transection of the cardiac branches of the vagi was performed. As a rule, no changes in cardiac activity were associated with this. Such results were obtained in 23 of the 30 experiments carried out; in 3 cases transient acceleration and in four – slowing of the heart rate were observed. Comparable results were obtained by A. G. Filippova (1940). The results of the 7 experiments in which denervation of the heart was associated with transient changes in the rhythm of cardiac contractions can be regarded as a consequence of the stimulating effect of the transection of the cardiac branches, particularly since in the previous series of experiments both acceleration and slowing of the heart rate under the influence of electric stimulation of these branches of the vagus had been observed by the present author. It is possible that the acceleration of the heart rate observed by Skramlik also arose under the influence of a definite degree of stimulation, determined by the method adopted by him for the transection of the branches of the vagus. Skramlik does not mention the duration of the changes associated with this. The factual material presented suggests that the presence of sympathetic innervation of the heart or of inhibitory tonus of the vagi is not obligatory for the appearance of a positive chronotropic influence on the heart. Nervous regulation of the rhythm of cardiac activity can be achieved in these animals by means of active vagal influences in the same way as that which has been found by M. G. Udelnov to prevail in higher vertebrates possessing sympathetic innervation of the heart.

Acceleration of the heart rate, as well as slight slowing, occurred on stimulation of the cardiac nerves in fish within a very narrow range of variation in the strength of stimulating current exceeding only slightly the threshold value. It was at times sufficient to alter the intercoil distance by fractions of a centimeter only to be able to observe the reversal of effect; further increase in the strength of stimulation led to temporary cardiac arrest which occurred over a very wide range of changes in current strength and this particular form of vagal influence was thus easily elicited under the given experimental conditions. It may be thought that prolonged cardiac arrest occurs very infrequently under normal physiologic conditions and that the changing requirements of the fish organism can be satisfied by relatively small changes of cardiac activity in one or the other direction. The ease with which extreme, one might say freakish, forms of nervous activity influence cardiac function may explain why Skramlik and other authors failed to observe acceleration of the heart rate in fish on electric stimulation of the peripheral ends of the vagus. Al the data presented contradict Skramlik's concepts regarding the mechanism of functional regulation of the heart in fish and permit the assumption that both inhibitory and stimulating effects of the nervous sytem on the heart rate in fish are achieved as the result of active vagal influences.

SUMMARY

The results of sixty experiments on bony fishes, mostly pike , with stimulation of the peripheral ends of the cardiac branches of vagal nerves are reported. Frequency of heart contractions varied in accordance with the intensity of stimulation: in one and the same experiment the heart would stand still, slow down and hasten its contractions. Bilateral section of the cardiac branches of vagal nerves would cause transient changes of heart contractions in some cases, but no change whatsoever in most of them. Skramlik's theory on the tonus of vagal nerves in fishes must be revised to include the possibility of an active positive chronotropic effect of vagal nerves.

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ANALYSIS OF THE HUMORAL BASIS UNDERLYING PERIODIC ALTERNATION OF CARDIAC INHIBITION AND STIMULATION ON PROLONGED STIMULATION OF THE VAGUS

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Present day data on the mechanism of the vagal effect on the heart indicate that the vagus can not only inhibit but also, under certain conditions, stimulate cardiac activity. Detailed studies of such dual influence of the vagus has led many scientists to conclude that the different nature of these different vagal effects is associated with secretion of chemically active substances during its stimulation [5, 6, 7, 8, 9, 10, 11].

In the light of the problem being considered in the present work of great interest are the researches of I. Went, E. Szues and E. Hetenyi [10], I. Went, E. Szues, T. Kovacs [11], M. Szentivanyi, A. Kover [12] and others which deal with the interrelations of acetylcholine and adrenaline. It has been shown that prolonged action of acetylcholine can promote liberation of adrenaline in the heart, i.e., acetylcholine stimulates cardiac activity by way of liberation of its antagonist – adrenaline. In the analysis of the humoral basis of vagal action on the heart a study of the Sechenov-Mechnikov phenomenon is especially relevant: this phenomenon consists of the fact that prolonged stimulation of the vagus (up to 40 minutes) elicits periodic changes in cardiac activity manifested by alternating inhibition and restoration of cardiac activity.

The present work is concerned with an attempt to find the answer to the question of whether the periodic alternation of inhibition and stimulation of cardiac activity observed on prolonged vagal stimulation is associated with a periodic appearance in the heart of chemically active substances which act differently on rhythmic cardiac activity during the different phases of vagal effect.

METHODS

All the present experiments were performed in accordance with the following scheme: three Straub-isolated frog (Rana temporaria) hearts were used. One of these – the donor heart – retained its connection with the medulla oblongata which was subjected to prolonged electric stimulation. It must be noted that both cardiac reactions (inhibition and restoration) occurred under identical experimental conditions, viz., with the same strength and frequency of stimulation. The heart was perfused with Ringer's solution; during definite phases of periodic cardiac activity under conditions of prolonged vagal stimulation the perfusate from the donor heart was transferred to the solution maintaining the recipient heart. Two recipient hearts were used in the experiment: one served as a testing agent for the phases of inhibition of the donor heart, the other for the phases of its restoration to full activity.

RESULTS

In the first series of experiments an attempt was made to clarify the question of whether it was possible for the Sechenov-Mechnikov effect to be mediated humorally. The results of these experiments are given in Fig. 1.

As kymogram I shows, prolonged stimulation of the vagus (31 minutes) elicits periodic alternation of inhibition and restoration of cardiac activity. Cessation of stimulation abolishes all forms of periodic activity.

Kymograms II and III show the results of experiments analyzing the possibility of "humoral transmission" of the periodic alternation of inhibition and restoration of cardiac activity upon prolonged stimulation of the vagus. As can be seen from kymogram II, the perfusate taken from the donor heart during a period of inhibition (a, c) causes a negative inotropic reaction in the recipient heart. Thus, perfusate (a), taken at the moment of the first arrest, diminishes the amplitude of cardiac contractions by 20%. Kymogram III shows the changes observed in the recipient heart treated with the perfusate collected from the donor heart during the phases of restoration (b, d, e). This perfusate causes a positive inotropic reaction in the recipient heart. Analogous data were obtained in all the other experiments of this series. The results thus indicate that the periodic vagal effect seen on prolonged stimulation of the nerve is accompanied by a periodic secretion of chemical agents which exert alternate inhibitory and stimulating influences on the heart.

The second series of experiments was devoted to a pharmacologic analysis of the nature of the substances liberated during the different phases of the periodic effect exerted on the heart by prolonged vagal stimulation.

The study of this question was begun by investigating the role of acetylcholine in the periodic appearance of inhibitory phases of cardiac activity on prolonged vagal stimulation. Experiments with proserine* and atropine were therefore carried out.

Action of proserine. These experiments were based on the hypothesis that proserine, being anticholinesterase preparation, should enhance the effect of the inhibitory substance appearing in the donor heart during the inhibitory phase if this substance were in fact acetylcholine. The experiments were performed as follows: proserine was introduced into both recipient hearts (concentration 1×10^{-5}). Perfusate taken from the donor heart during inhibitory and restorative phases was then tested on these hearts. The results obtained in this series of experiments are given in Fig. 2.

Kymogram III shows that proserine promotes a considerably more profound negative inotropic effect of perfusate collected from the donor heart during phases of inhibition (a, c, e, f, h). For instance, perfusate (a), taken at the moment of the first arrest of the donor heart, decreases the amplitude of cardiac contractions by 90% with simultaneous slowing of the rhythm by 25% as compared with normal; in the second phase of inhibition of the donor heart the perfusate collected from it (c) again exerts strong inhibition on the recipient heart, decreasing the amplitude of cardiac contractions by 62.5% this time. Analogous data were obtained throughout the duration of the whole experiment (kymogram III, e, f). Analysis of the effect of perfusate collected from the donor heart during the phase of restoration (Fig. 2, kymogram II, b, d, g) revealed that the response reaction of the recipient heart was unaltered; the perfusate in this case elicited the usual positive inotropic effect.

Treatment of recipient hearts with an anticholinesterase preparation – proserine – thus leads to marked deepening of the effect exerted by the "inhibitory" substance liberated by the donor heart during the phases of cardiac arrest. The effect of perfusate taken from the donor heart during the inhibitory phases is increased four-fold as compared with the effect of the same perfusate on the recipient heart which had not been treated with proserine; at the same time the effect of the "restorative" perfusate elicits the usual positive inotropic cardiac reaction. This fact appears to the present authors to indicate that periodic arrest of myocardial activity associated with prolonged vagal stimulation depends on the presence of acetylcholine. During the phases of restoration substances different in nature are probably liberated; this question is to be considered more fully below.

In the next series of experiments an attempt was made to discover the effect of atropine on the response of the recipient heart to the action of perfusate from the donor heart; atropine was chosen as a substance possessing properties abolishing the effect of acetylcholine on the one hand, and not interfering with the action of adrenaline on the other [4].

The effect of atropine. The experimental conditions were the same as in the preceding series except that the recipient hearts were treated preliminarily with atropine (concentration 1×10^{-5}).

Atropine abolishes the negative inotropic effect: perfusate taken from the donor heart at the moment of the first arrest does not alter the amplitude of contractions of the recipient heart (3.7-3.6 cm); using perfusate taken at the moment of the second arrest also leaves the amplitude of recipient heart contractions unchanged (3.5-3.5 cm).

* Russian trade name.

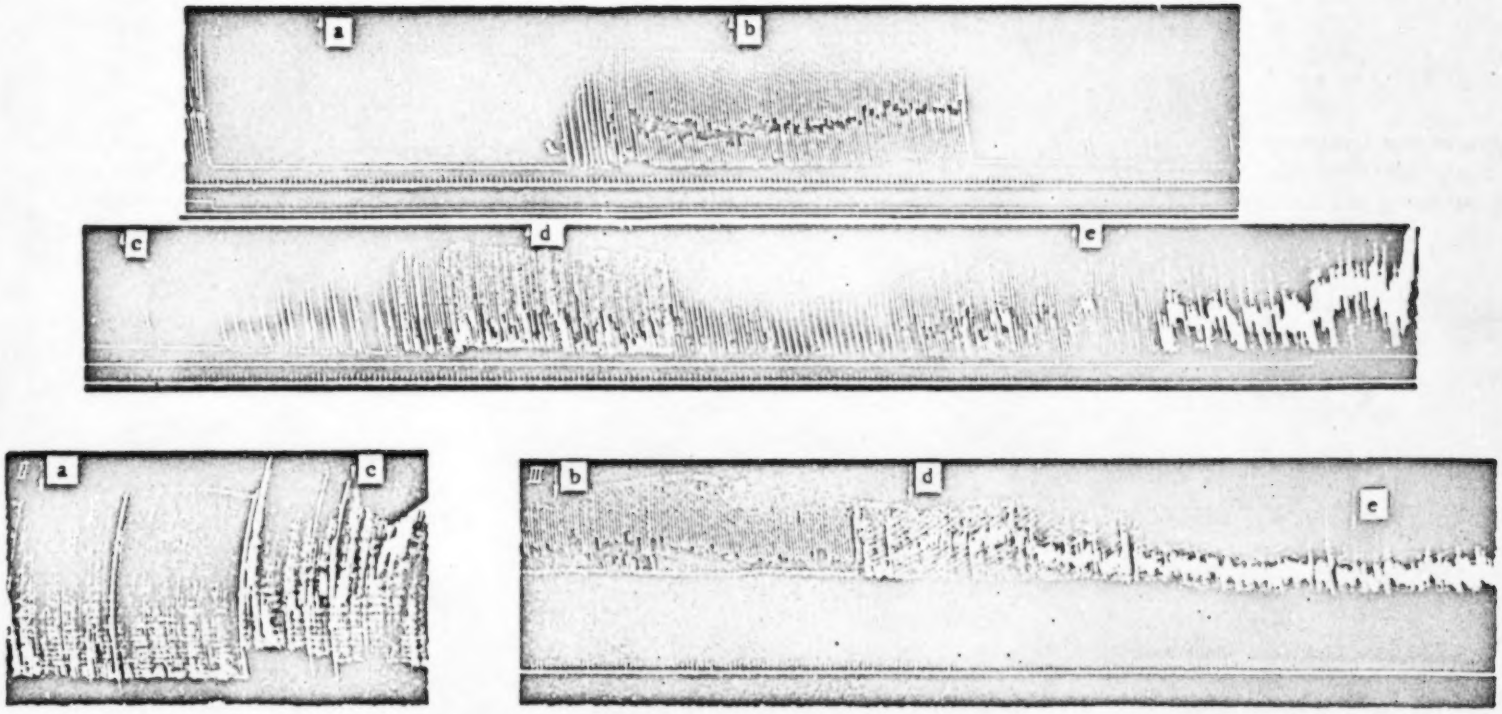
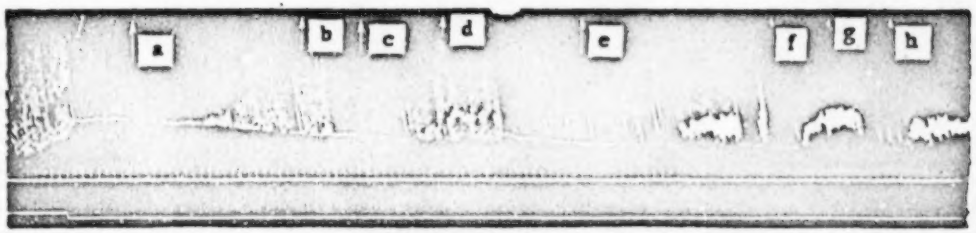
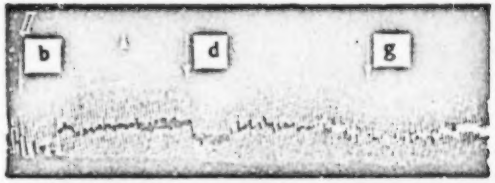


Fig. 1. I) Periodic alternation of inhibition and restoration of activity of the donor heart on prolonged vagal stimulation (duration of stimulation 31 minutes, strength 6 V, frequency 20 cps; II) changes in activity of the recipient heart receiving perfusate from the donor heart during the inhibitory phases; III) changes in activity of the recipient heart receiving perfusate from the donor heart during the restorative phases. The time marker is common for the recipient hearts; letters and arrows in all cases denote the moment of perfusate withdrawal from the donor heart and its transfer to the corresponding recipient hearts.





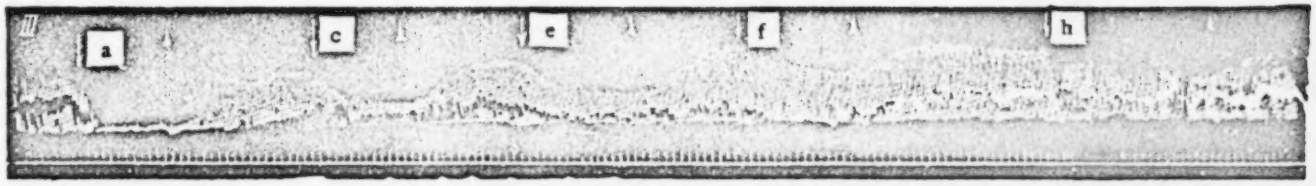


Fig. 2. I) Periodic alternation of inhibition and restoration of the activity of the donor heart on prolonged vagal stimulation (duration – 12 minutes, strength 5 v, frequency 20 cps; II) changes in the recipient heart activity on receiving perfusate from the donor heart during phases of restoration; III) changes in activity of recipient heart receiving perfusate from the donor heart during phases of inhibition. Both recipient hearts treated preliminarily with proserine (1×10^{-5}).

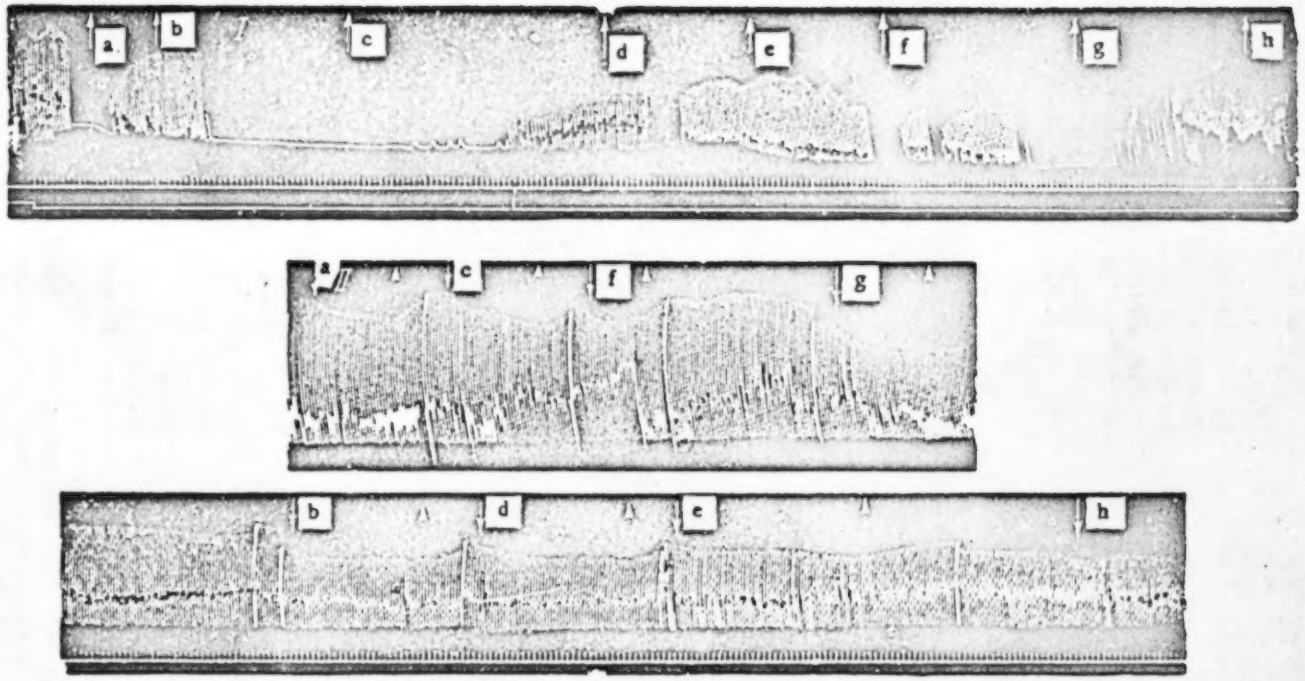


Fig. 3. I) Periodic alternation of phases of inhibition and restoration of the donor heart on prolonged vagal stimulation; II) changes in the activity of recipient heart receiving perfusate from donor heart during restorative phase; III) changes in recipient heart activity upon receiving perfusate from donor heart during inhibitory phases. Both recipient hearts subjected to preliminary treatment with dibenamine (1×10^{-5}).

Examination of the action of perfusate collected from the donor heart during the phase of restoration revealed that the response of the recipient heart was usually positively inotropic in character.

Treatment of recipient hearts with atropine thus leads to abolition of the negative inotropic effect caused by perfusate collected during the phases of inhibition of the donor heart, while the reaction of atropinized recipient hearts to the action of "restorative" perfusate preserves the usual positive inotropic character of the response.

It follows from all the experiments carried out that during the different phases of cardiac activity under the influence of prolonged vagal stimulation substances of different nature are apparently liberated. This conclusion is supported by the fact that both proserine and atropine change the character of the effect exerted by the substance liberated during the inhibitory phase but do not change the effect of the substance secreted during the restorative phase of the donor heart. The data cited above suggest that the agent acting during the inhibitory phases is acetylcholine, while during the restorative phases adrenaline-like substances are most probably liberated. To test this hypothesis a series of experiments with dibenamine – an antisympathetic and anti-adrenaline agent were performed.

The effect of dibenamine. Experiments with dibenamine were carried out as follows: dibenamine (concentration 1×10^{-6})* was introduced into both recipient hearts and the action of perfusate from the donor heart collected during the phases of inhibition and restoration was then tested on these.

As can be seen from the kymogram (Fig. 3, kymogram III, b, d, e, h) dibenamine abolishes the usual positive inotropic effect of perfusate collected from the donor heart during the inhibitory phase. Thus, perfusate (b), taken at the moment of the first period of restoration of the donor heart does not exert the usual positive inotropic effect; on the background of dibenamine action a similar effect is produced by perfusate taken from the donor heart during subsequent phases of restoration – b, d, etc. Examination of the action of perfusate collected from the donor heart during inhibitory phases (Fig. 3, kymogram II, a, c, f, g) showed that the response of dibenamine-treated recipient heart was the same as that of a normal recipient heart: the perfusate in this case evoked the usual negative inotropic effect.

Treatment of recipient hearts with an anti-adrenaline agent – dibenamine – thus leads to the abolition of the positive inotropic reaction occurring in response to the introduction of perfusate collected from the donor heart during restorative phases. The slight diminution in the amplitude of cardiac contractions as compared with the normal, observed in these experiments can be explained either by the presence of a certain amount of acetylcholine in admixture with the adrenaline-like substances in the "restorative" perfusate and the inactivation of these substances by dibenamine with consequent "unmasking" of the action of admixed acetylcholine (Baker, 1954), or by a distorting effect exerted by dibenamine on the action of adrenaline (Zakusov, 1953).

As a control, all the experiments mentioned above were performed on hearts which had been freed from the influence of the sympathetic nervous system. Experiments with such hearts (on the 15th day after denervation) showed that prolonged vagal stimulation, in the absence of sympathetic nervous system influences, was associated with periodic alternation of inhibitory and restorative phases, with "humoral transmission" of this effect.

These experiments, therefore, lead to the following conclusion: functional, periodic changes in the heart muscle which accompany prolonged vagal stimulation (uniform in strength and frequency) are determined by the periodic appearance of chemical substances of different nature: acetylcholine during the phases of cardiac inhibition and adrenaline-like substances during the phases of restoration.

We cannot as yet, on the basis of our data, determine the mechanism of the appearance of these substances during definite phases of cardiac activity. In accordance with the data found in literature [10, 11] the most likely hypothesis is that acetylcholine, which is liberated during stimulation of the vagus, upon reaching a certain degree of accumulation and a certain stage of interaction with the physico-chemical receptor systems promotes the liberation of substances with a stimulating action similar to that of adrenaline-like agents. Acetylcholine, in the opinion of the Oxford school of pharmacologists [5] plays the role of regulator of the rhythmic activity of the heart; it probably plays this part during prolonged vagal stimulation also when periodic alternation of phases of inhibition and restoration of rhythmic cardiac activity are observed.

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* This value contradicts the one given in Fig. 3 caption. – Publisher.

SUMMARY

Prolonged stimulation of the vagus nerves (up to 40 minutes) evoked periodic changes in the activity of the heart muscle. It is manifested by the interchange of phases of inhibition and recovery of heart activity (Sechenov-Mechnikov phenomenon).

Experiments were performed on hearts of frogs (Rana temporaria). It was established that the functional periodicity in the activity of the heart, which was observed under conditions of prolonged stimulation of the vagus nerves is associated with periodic appearance of chemically active substances of different origin in the heart. Thus, the periodic appearance of phases of inhibition is connected with periodic secretion of acetylcholine, while the periodic apperance of phases of recovery – with periodical secretion of adrenaline-like substances.

It is probable that acetylcholine plays a regulating role in cardiac activity, promoting liberation of adrenaline-like substances under conditions of prolonged stimulation of the vagus nerve.

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CONCERNING THE INFLUENCE OF THE BRAIN CORTEX ON CAPILLARIES

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According to the data of V. N. Chernigovsky [5], T. A. Grigoryeva [3] and others, the capillary bed is the reflexogenic field for many vascular and other visceral reflexes. T. A. Grigoryeva believes that the receptors located in the capillary bed are the tissue exchange receptors.

There are a series of works describing how the action of different stimuli on the capillaries and on their surrounding tissue changes the normal state of the capillaries.

O. A. Balandina [1] has indicated the presence of neuroreflex regulation of the capillary system.

We could find very little literature (except for V. A. Bondina's work [2]) concerning the reflex change which occurs in capillary lumen with the stimulation of remote receptor zones.

The purpose of this work was to study the influence of the cerebral cortex on the condition of capillaries. We examined both unconditioned and conditioned reflex changes in capillary lumen, caused by slight, painful stimulation of cutaneous receptor zones remote from the experimental region in order to effect this study.

EXPERIMENTAL METHODS

We examined four, generally healthy women, from 25 to 35 years in age, by using the method of capillaroscopy on the region of the nailbed of the third finger of the right hand. Painful stimulation was applied by means of a light prick with a needle near the base of the fifth finger of the left hand. A metronome with a frequency of 120 beats per minute was used as the conditioned stimulus. The experimental subjects were separated from the experimenters by two screens, one on each side, so that stimulation and capillaroscopy could be done unseen by the subjects.

The subjects were questioned as to their general condition before each examination. The original condition of the capillaries was first determined according to an established plan – the background, the number of capillaries in the main and most clearly evident group, the shape and tonicity of the capillaries and the character of the bloodstream. After stimulation had been applied, we determined how all the above-enumerated factors had changed, especially the tonicity and number of the capillaries, the latent period of the reaction, the duration of the reaction and the time of restoration. The reaction intensity was determined (expressed in pluses – one plus, two plus, etc.) from the sum total of all these changes. The stimuli were given 6-8 times in each experiment at intervals of 3-5 minutes varying in order to prevent the development of a conditioned reflex to time.

EXPERIMENTAL RESULTS

A light prick near the base of the third finger of the right hand caused the capillaries to constrict sharply (3-4 plus) and their quantity to decrease from 12-14 in the main group to 8-10 in all four subjects. The reaction lasted from 5 to 15 seconds depending on the force of the stimulus. Restoration of original capillary condition took from 5 to 15 seconds, also depending on the force of the stimulus applied. The latent period of the reaction was 3-5 seconds.

When the prick was applied to the opposite hand, the same changes occurred in the capillaries (Fig. 1).

There was no specific difference between the right and left hand in the capillary reaction to the applied prick. The force of the stimulus, as in the previous case, affected the intensity of the capillary reaction and the quickness of restoration. In several cases, restoration of the capillary reaction was protracted and fluctuating when strong stimulation was applied to both hands. We did not observe in the daily examinations any adaptation to the pain stimulus either during the days of observation or towards the end of each experiment. The reaction remained the same.

We include a detailed case history of one experiment in this series (see Report of Experiment 3).

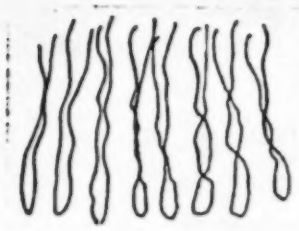

Fig. 1. Reflex constriction and quantity decrease of capillaries caused by the action of the unconditioned stimulus applied to the opposite hand. (All figures show only the main and most clearly evident group of capillaries).

Having concluded from the above experimental series that there is a reflex mechanism for capillary regulation, we began to develop in the subjects a conditioned reflex to the sound of a metronome with a frequency of 120 beats per minute, which was combined with a needle prick near the base of the fifth finger of the left hand. The capillary reaction to the action of the metronome was first erased. The first three combinations were done simultaneously, but later, the unconditioned stimulus was separated from the conditioned by 5 seconds. Conditioned reflex capillary constriction appeared in all the subjects after the 4th-5th combination and thereafter was observed in almost all the combinations. Capillary constriction, increased capillary tonicity and a decrease in capillary quantity from 12-14 to 6-10 were observed with the action of the unconditioned stimulus even before it was reinforced by the unconditioned, painful stimulus. The reaction intensity was recorded by 2-3 plus; the duration of the reaction was from 5 to 12 seconds; and the restoration time was 5 to 13 seconds. In other words, we obtained the capillary changes as with the unconditioned stimuli. According to the degree to which the conditioned reflex had been developed, the latent period of the conditioned reaction was somewhat shorter in all of the subjects than the latent period of the unconditioned capillary reaction and was 2-3 seconds long. The action of the conditioned stimulus alone, without the prick reinforcement, caused similar changes (Fig. 2).

Therefore, we were able to develop a conditioned reflex to change in capillary lumina, and this fact indicates that the cerebral cortex has a definite influence on capillary activity. In order to further examine the influence of the cerebral cortex on capillaries, we developed a differentiation and a capillary reaction to a verbal stimulus and examined the way in which the conditioned reflex was erased.

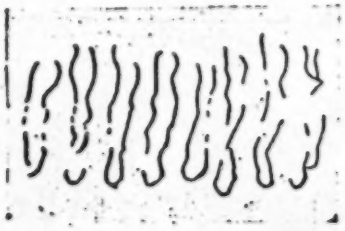

Fig. 2. Conditioned reflex constriction and decrease in quantity of capillaries in response to M_{120} stimulus (metronome with a frequency of 120 beats per minute).

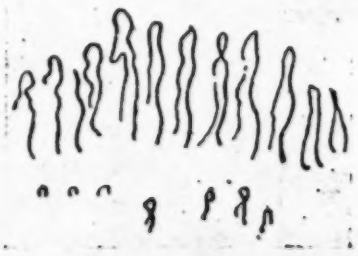

Fig. 3. Absence of capillary reaction to differentiation stimulus M_{60}.

Experimental Record No. 3, October 17, 1954, Subject – B

Time	Stimulus	No. of stimulus	Latent period (in seconds)	Condition of capillaries					Reaction intensity	Reaction duration (in seconds)	Restriction time (in seconds)
				Number in main group	Background	Shape	Tonicity	Bloodstream			
10:40	—	—	—	14	Pink	Convoluted	Normal	Homogeneous	—	—	—
10:45	Prick	1	4	10	"	"	Increased	"	+	8	12
10:50	"	2	4	10	"	"	"	"	+ +	10	12
10:57	"	3	5	10	"	"	"	"	+ + +	10	10
11:00	Light prick	4	5	10	"	"	"	"	+ +	10	10
11:04	Strong prick	5	3	8	"	"	"	"	+ + +	13	17+15 Fluctuating

Experimental Record No. 9, October 25, 1954, Subject – B

Time	Stimulus	No. of stimulus	Distance apart (in seconds)	Latent period (in seconds)	Condition of capillaries					Reaction intensity	Reaction duration (in seconds)	Restoration time (in seconds)
					No. in main group	Background	Shape	Tonicity	Bloodstream			
9:18	—	—	—	—	14	Pink	Convoluted	Normal	Granular	—	—	—
9:20	M_{120} + Prick	38	5	3	12	"	"	Increased	"	+	6	7
9.25	M_{120} + Prick	39	5	3	12	"	"	"	"	+ +	7	5
9:27	M_{60}	10	—	—	14	No reaction						
9:29	M_{60}	11	—	—	14	The same						
9:30	M_{60}	12	—	—	14	"						
9:31	M_{120} + Prick	40	5	6	10	Pink	Convoluted	Increased	Grainy	+ +	5	5
9:36	M_{120} + Prick	41	5	6	12	"	"	"	"	+ +	6	5
9:40	M_{120} + Prick	42	5	3	12	"	"	"	"	+ + +	5	
9.50	M_{120} + Prick	43	5	3	12	"	"	"	"	+ + +	5	4

As a differentiation stimulus, we used a metronome with a frequency of 60 beats per minute, not reinforced by a prick. The first few uses of this stimulus caused the same capillary changes as the M_{120} stimulus with the prick reinforcement, which fact seemed to us to indicate the generalization of the conditioned reflex. After the 6th-8th use, M_{60} caused no change in capillary condition (Fig. 3), while M_{120} continued to cause the changes already mentioned.

Analyzing this experiment, we not only observed a complete lack of capillary reaction to the differentiation stimulus but also a subsequent inhibition, which appeared after the third use of the differentiation stimulus and was expressed by an absence of the conditioned reaction to two conditioned stimuli used after the differentiation; the capillary reaction only appeared after reinforcement.

When the second signal system of stimuli, consisting of a verbal warning – " I am pricking " – without reinforcement by the unconditioned stimulus, caused a sharp capillary reaction, exceeding that which occurred in response to the combination "M_{120} + prick." The use of indifferent verbal stimuli – window, table, wire, etc. – did not cause any change in the capillary condition of the subjects.

Repeated nonreinforcement of M_{120} with a prick caused the intensity of the capillary reaction to gradually decrease until the complete and permanent disappearance of the reaction after 10-15 uses of the nonreinforced M_{120}. The erasure of the conditioned reflex fluctuated; along with the lack or sharp decrease in the capillary reaction to M_{120} during the erasure period, capillary constriction was sometimes quite pronounced.

Therefore, all the investigations conducted indicate that human capillaries react sensitively by conditioned and unconditioned reflexes to various environmental stimuli associated with the first and second signal systems and that they are always under the constant control of the cerebral cortex.

SUMMARY

Experimental data are presented on the presence of unconditioned and conditioned capillary reflexes to various stimulants, pertaining to the first and second signal system. It has been established that if the left wrist is stimulated, there is reflex constriction of capillaries of the right wrist. It appeared possible to elaborate a conditioned reflex of capillaries to pain stimulation with differentiation. Verbal stimulation, such as: " I'm pricking", evoked more pronounced reaction of capillaries than unconditioned or conditioned stimuli.

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INTEROCEPTIVE CONTROL OF BLOOD COAGULATION

COMMUNICATION I. THE EFFECT OF REFLEXES FROM THE CAROTID SINUS CHEMORECEPTORS ON BLOOD COAGULATION

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Nervous regulation of blood composition was first suggested by S. P. Botkin in 1875. Exteroceptive influences on the process of blood coagulation have been established by many authors [6, 3, 2, 8]. Recently, A. A. Markosyan [4] has established the possibility of conditioned reflex change in the rate of blood coagulation.

Interoceptive regulation of the morphological composition of the blood has been examined in works by A. Letner [9], V. N. Chernigovsky and A. Ya. Yaroshevsky [5] and others. Interoceptive regulation of the blood-coagulating system has not been investigated at all to date, unless the work of E. Perlik [11], studying the effect of reflexes from the carotid sinus pressoreceptors on certain aspects of blood coagulation, is counted, although the author did not treat coagulation as a whole.

Even before Perlik's article appeared, we had finished this present study, the purpose of which was to establish the possibility that blood coagulation is regulated by the reflexes from the carotid sinus chemoreceptors. The resolution of this question could shed some light on the pathogenesis and prophylactics of thrombo-embolic disease, which is now a question of real importance to practical medicine.

EXPERIMENTAL METHODS

24 experiments were done on dogs. The experiments were conducted under morphine-pentothal anesthesia. After tracheotomy, we began to prepare a preparation isolated vascularly, but with the nerve links of the carotid sinus angioreceptive field preserved by the method of Moiseev-Geimans-Anichkov. For this, all the vessels of the sinus region were ligated, while the nerve fibers were preserved and, as far as possible, left unharmed. A glass cannula was inserted into the peripheral end of the common carotid artery in order to introduce the Tyrode's solution, and a second, withdrawing cannula was inserted into the central part of the lingual artery. Perfusion was done at a rate of 50-60 drops per minute. The Tyrode's solution rinsing the sinus entered under 125 cm of water pressure, through a tube, into a coil placed in a water bath 38-40° in temperature; from the coil, the heated solution entered the sinus.

After the preparations were finished, the original rate of blood coagulation was determined before permanent results were obtained. Blood for examination was taken from the lower part of the femoral artery by our modification of Kennon's method. This method makes it possible to obtain fresh blood by means of paraffined cannulas without changing the properties of the blood and without the admixture of extraneous tissue elements, which occurs with the use of a hypodermic or pricking, which may alter the true rate of blood coagulation. The moment of the entrance of the blood into the cannula was recorded with a stopwatch; coagulation time was determined by Fonio's method.

After the original level of the blood coagulation rate had been determined, 0.25-2 ml of acetylcholine or nicotine, in dilutions of from 10^{-4} to 10^{-7} and preliminarily heated to the temperature of the perfusion fluid,

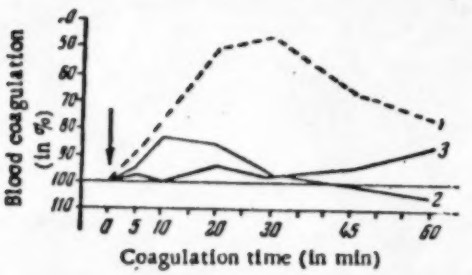
were introduced into the perfusion current (once, in some experiments, in others, two or three times). We gauged the preservation of the carotid sinus nerve links according to the reflex change in respiration, which was recorded on a kymograph tape.

The blood coagulation rate was again determined at intervals of 5, 10, 20, 30, 45, 60 and, in some experiments, 90 minutes after stimulation of the carotid sinus chemoreceptors.

EXPERIMENTAL RESULTS

In the first series of experiments, we examined blood coagulation after the nicotine and acetylcholine solutions had been introduced into the current perfusing the carotid sinus. Both stimulants gave approximately the same results. A more or less expressed shortening of blood coagulation time was observed in all cases. From the average curve shown in the graph, one can see that the coagulation time decreased by almost 50% after stimulation of the chemoreceptors. The greatest coagulation acceleration was obtained 20-30 minutes after stimulation and lasted about 1-1½ hours.

To prove the reflex character of the results we obtained, we conducted a second, control series of experiments with denervation of the carotid sinuses, in which the sinus nerve was cut, and the afferent part of the reflex are thereby broken. In these experiments, no reflex change in the blood coagulation rate was obtained in response to the introduction of nicotine or acetylcholine into the perfusion current (see Figure).



Blood coagulation rate under different conditions: carotid sinus chemoreceptors stimulated with a solution of nicotine or acetylcholine (1), denervation of suprarenal glands (2), denervation of the carotid sinus (3).

In the same way, no change in the blood coagulation rate was observed with perfusion of the carotid sinus, with the sinus nerve intact, without the introduction of the chemical stimulants. As a rule, however, the coagulation time had gradually decreased by the end of the control experiments. This was evidently connected with the effect of the narcosis and the release of thromboplastin from the wounded tissues.

Starting from the works of Kennon [6], M. S. Klimova [3] and others, in which the influence of suprarenal gland adrenalin secretion on blood coagulation was shown, it was interesting to determine the importance of the adrenal system to the realization of the blood coagulation reflex from the carotid sinus chemoreceptors. For this purpose, the celiac nerves which are the secretory nerves for the suprarenals were transected under the diaphragm.

These experiments showed that, under conditions of suprarenal gland denervation, blood coagulation is very little affected by stimulation of the carotid sinus chemoreceptors, although this causes a marked respiratory reflex. In the experiments, blood coagulation time had decreased an average of 17.2% by the 10th minute, but then increased again, even exceeding the original level (by 4.7%, see graph) one hour after stimulation of the carotid sinus chemoreceptors.

The facts we have presented regarding the reflex acceleration of blood coagulation are especially interesting in connection with clinical observations on the relationship of unexpectedly developing thrombosis and the stimulation of sinocarotid zones of heightened excitability [7, 10]. Since nicotine caused the acceleration of blood coagulation, one must consider smoking to be forbidden to people threatened with thrombotic complications: such people include patients during pre- and postoperative periods, those with preinfarction conditions or diseases of the cardiovascular system, etc.

SUMMARY

Nicotine or acetylcholine stimulation of the carotid sinus in dogs shortens the coagulation time of the blood. The time of clotting is the shortest 20-30 minutes after the start of stimulation. Perfusion of the carotid sinus with the sinus nerve intact and no stimuli applied, as well as the injection into the denervated zone of nicotine or acetylcholine caused no reflex change of the clotting time. Experiments with the celiac nerve indicate

that the secretion of adrenalin by the adrenal glands is an important, though not the sole factor in the reflex effect of the carotid sinus chemoreceptors on the blood coagulation time.

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THE EXCITATION WAVE ANALYZED AS A PARABIOTIC PROCESS

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N. E. Vvedensky [2] considered the theory of parabiosis which he worked out on a neuromuscular preparation to be a master mode of phenomena occurring in all sections of the nervous system. Further studies [1,5,6] experimentaly confirmed the validity of Vvedensky's basic conception concerning the importance of steady state excitation to the activity of the central nervous system. A series of researchers including L. L. Vasilyev [1], A. N. Magnitsky [6] and especially L. V. Latmanizova [5] found that many signs common to both steady and spontaneous stimulation appear during the spread of an excitatory wave.

This is a reason for regarding the stimulation wave as a peculiarly proceeding parabiotic process. We agree with L. V. Latmanizova that complete parabiosis develops immediately upon the appearance of a stimulatory wave and that it almost as immediately ceases, the stimulated portion of the organ then gradually passing through the stages of the parabiotic process in the reverse direction. It has been established that the relative duration of the parabiotic process depends on the agent used. With the action of some stimuli (anode of a continuous current, $CaCl_2$, heat, etc.), the first phase of parabiosis development is extraordinarily long, and the transition of the stimulated part into the second, or heightened excitability, stage is very slow (A in Fig.1). The action of other agents (cathode of a continuous current, KCl, cold, etc.) produces a short first phase and a very long second, or catelectrotonic, phase (B in Fig. 1). One can propose that the first two phases of the parabiotic process are extraordinarily brief with the development of a stimulatory wave, the inhibition condition occurring almost immediately (C in Fig. 1), after which, but more slowly, the substrate returns to its original condition by passing through the same stages in reverse order.

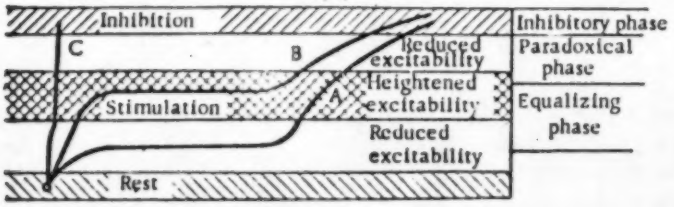

Fig. 1. Development of the parabiotic process under the influence of: A) anode of a continuous current; B) cathode of a continuous current; C) superthreshold stimulation.

When one compares the electric phenomena attending a single impulse with this process (Fig. 2), it appears that the maximal electronegativity, i.e., the peak of the action potential (point a) corresponds to the developed parabiosis, while the ensuing changes of the action potential will correspond to the stages of the

reverse parabiotic process. The rapid fall of the high-voltage potential corresponds to the parabiotic process stage of profound inhibition, with its characteristic sharp decline of excitability. The negative after-potential period reflects the paradoxical and equalizing stages; excitability rapidly increases during this period – it is lower than the original (relative refractability) at first, but then exceeds it (heightened excitability or exaltation stage). The positive after-potential period corresponds to the first, or reduced excitability stage of the parabiotic process.

If the excitatory wave is considered to consist of parabiosis developing immediately and as immediately checking to pass through all the stages described above in reverse order with ever-decreasing speed, the features of the biological substrate's rhythmic reaction caused by both the substrate's inherent activity and by artificial stimulation can be explained.

EXPERIMENTAL METHODS AND RESULTS

We succeeded in proving the parabiotic nature of the phenomena developing during a stimulation wave in experiments conducted on animals and people in which we induced rhythmic excitation by continuous current impulses of a definite duration. With square wave impulse generator of variable duration and frequency, we used indirect, rhythmic stimulation of the muscle in such a way that the effect of each shock of current would consist of useful time, i.e., of the initial stimulation effect, causing the development of an impulse, and of the

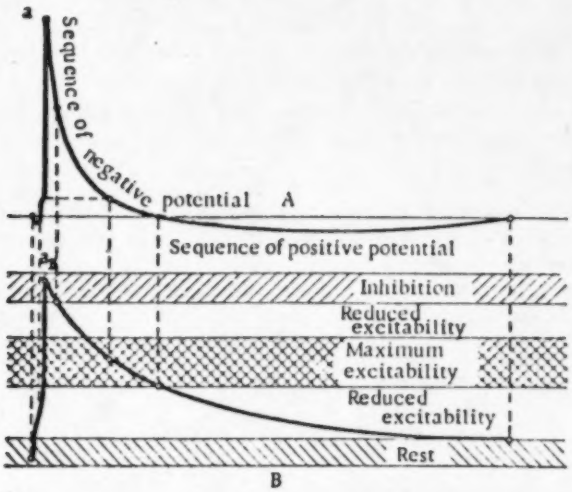

Fig. 2. Scheme illustrating comparison of electrical effect (A) in path of parabiotic process (B) in course of a single wave stimulation.

subsequent effect, not causing a new excitation wave to appear. As the continuous current action included this second phase, which lasted a very brief, exactly computed period of time, it can be considered to correspond to the introduction of a parabiotic agent [2,3,4,7,8,9]. In other words, with such an experimental setup, the rhythmic introduction of brief continuous current shocks not only causes the appearance of an impulse, but also affects the restorative processes. Our aim was to establish what effect different durations of these shocks had on the magnitude of the frequency threshold of muscle partial pessimum, which effect was estimated by the initial decrease in the contraction in response to an increased frequency of stimulating impulses.

In each of the 15 short experiments which we conducted on cats by means of indirect stimulation of the anterior tibialis muscle, the frequency threshold caused by stimulating impulses lasting 0.5, 1, 4 and 8 sec was determined several times. In all of the experiments, without exception, the frequency required to maintain maximal contraction with short impulses was comparatively high, but the longer the impulses were, the lower the frequency causing contraction height to diminish. It was clearly demonstrated that the longer the stimulating impulses, the greater the pause between them should be in order to maintain the maximal reaction. The total results of these experiments are summarized in Table 1 in which each figure shown for the thresholds and pauses represents the average from 50-60 observations.

Similar results were obtained from observations on people, recording the movements of the thumb in response to stimulation of the distal motor point of the median nerve, with stimulation impulses from 1 to 8 m sec in duration. However, with all of the experimental durations, a considerably greater frequency was required to obtain partial pessimum in the people than was required in the cats. It also appeared that the threshold of partial pessimum changed with age in humans. Table 2 gives the average amounts from 65 observations conducted on 12 subjects, varying from 25 to 30 years of age. We observed a similar picture, but with lower frequencies, in children of different ages and in adolescents.

TABLE 1

Experiments on Cats (Average Amounts)

Frequency threshold of partial pessimum	Duration in msec		
	Impulse	Pause	Total period (impulse + + pause)
135	0.5	6.91	7.41
103	1	8.71	9.71
60	4	12.67	16.67
46	8	13.74	21.74

TABLE 2

Observations on Adult Humans

Frequency threshold of partial pessimum	Duration in msec		
	Impulse	Pause	Total period (impulse + + pause)
466	1	1.14	2.14
259	2.5	1.36	3.86
125	4	4.00	18.00
77	8	4.99	12.99

The data presented indicate that increasing the duration of the stimulating impulses retards the restoration of the organ to the original state, i.e., decreases the rate of reverse parabiosis. The graphs in Figure 3 illustrate the reverse course of the parabiotic process during the time interval between two alternating stimulation impulses in which the pessimal reaction was first observed.

The upper graph represents the experiments with animals, the lower, those with people. The line AF, in the upper graph, represents the lower boundary of the inhibition stage, or the moment of absolute refractability termination. The point O represents the parabiosis immediately appearing under the influence of the alternating stimulation impulse. OC_1E_1, OC_2E_2 etc. are the restoration curves, i.e., show the reverse course of the parabiotic process with different durations of the stimulating impulses and intervening pauses. The impulse durations are shown by the sections AB_1 (0.5 m sec), AB_2 (1 m sec), AB_3 (4 m sec), AB_4 (8 m sec), and the pause durations by the sections B_1E_1 (6.91 m sec), B_2E_2 (8.71 m sec), B_3E_3 (12.67 m sec), and B_4E_4 (13.74 m sec).

Therefore, the left portion of each restoration curve (sections OC_1, OC_2, etc) represents the action period of the continuous current, and the second portion (sections C_1E_1, C_2E_2, etc) represent the pause period.

Since the 0.5 m sec duration of the impulse is actually only net stimulation time, one can consider the curve $O_1C_1E_1$ to show the speed of the reverse parabiotic process uncomplicated by the effect of the continuous current. The longer stimulating impulses, however, have a dual effect: they cause stimulation and have a parabioticizing effect, i.e., retard the reverse course of the parabiotic process. The protracted action of a continuous current gradually weakens its parabiotic effect due to the phenomenon of accomodation, as the graph shows (gradual descent of curve OC_1, C_2,C_3,C_4). Moreover, the longer the action of the continuous current, the greater the time needed for the restoration process: $C_4E_4 > B_3E_3 > B_2E_2 > B_1E_1$.

Similar results were obtained from the observations on people, as can be seen from the lower graph in Figure 3, constructed in the same way as the upper graph.

Comparing the two graphs, the first thing one notices is that the rate of the reverse course of the parabiotic process is considerably faster in people than in cats. This is in conformance with the greater lability of human muscles.

In this way, starting with the conception of the stimulation wave as an immediately developing, reversible, parabiotic process, we have attempted to explain how the duration of the stimulating impulses affects the character of the organ's responding reaction.

Instead of the metaphysical principle, "all or nothing", or, in other words, the principle concerning the alternation of assimilation and dissimilation (and therefore, the presence and lack of activity and the presence

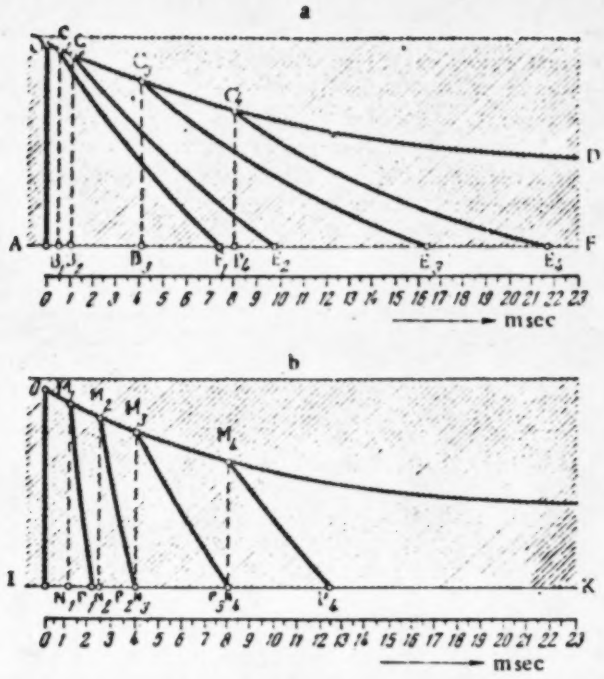

Fig. 3. Speed of reverse parabiotic process in relation to duration of stimulating impulses.
a) experiments on cats; b) observations on humans.

and lack of ability to react to stimulation) as a conclusion reached from the position that a live organ always reacts to stimulation, we propose the presence of the reversible parabiotic process. Excitability is only a particular phase of the reactivity of live matter, just as wave stimulation is a particular phase of an active, energetic condition.

A stimulated substrate reacts in response to any stimulating action by a single parabiotic process, but the form of this process can vary; in some cases, it develops in a wave-like manner, in others, in the form of a local, nonfluctuating process.

SUMMARY

The aim of this study was to verify the hypothesis that the excitatory wave is a reversible parabiotic process. Experiments were performed on people and cats. A nerve was subjected to serial frequent short stimuli by a constant current for 0.5, 1.4 and 8 msec. and the frequency of stimuli resulting in the appearance of a pessimal muscular reaction was determined. Pessimum appeared at much lower frequencies if stimuli were prolonged – the pause between stimuli being larger. This phenomenon is considered as a delayed reversible course of the parabiotic process, caused by the constant current.

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THE ROLE OF THE STOMACH RECEPTORS IN GASTRIC SECRETION

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It has been established in the laboratory of I. P. Pavlov [1] that the greater the amount of food intake, the more gastric juice is secreted. Secretion duration increases approximately $1\frac{1}{2}$ times when the amount of food consumed is doubled. These facts have been proved [2, 3]. In the studies cited, the amount of food consumed did not cause the animal to become completely satiated; on the contrary, the animals greedily consumed all food placed before them.

It was also observed in the laboratory of I. P. Pavlov that the consistency of food has a definite influence on gastric secretion [4].

Gastric secretion in dogs with a Pavlov stomach has recently been studied under conditions of meat fed in small amounts many times until complete satiation was obtained [5]. It was found that juice secretion was maximal 30-40 minutes after the first small feeding, and thereafter decreased.

Until recently, there was no one opinion on the role of the gastric mechanoreceptors in gastric secretion. Only thanks to carefully conducted studies [6] and [7] has the question of the mechanical factor as a stimulator of gastric secretion been conclusively and positively solved. Further studies [8] have shown that strong mechanical stimulation of the gastric walls sharply inhibits gastric secretion caused by simulated feeding.

There are also indications in the literature that strong stimulation of the stomach receptors causes both conditioned and unconditioned reflexes to be inhibited [9, 10, 11, 12, 13, 14].

In this article, we present experiments studying the secretory activity of the salivary and gastric glands caused by feeding the animal different amounts of food or by expansion of the gastric walls to different degrees.

EXPERIMENTAL METHODS

Dogs were used in the experiment: 3 with a stomach isolated according to Pavlov, 2 with a stomach isolated according to Klemensevich-Heidenhain and 3 with a permanent salivary gland fistula, stomach isolated according to Pavlov and Basov's gastric fistula.

The gastric juice was collected for periods of 4,5 and 10 hours, the free hydrochloric acid and the total acidity in the hourly batches were determined by the titration method, and the peptic activity in the total amount of juice and, in a series of experiments, the dry residue with its organic and inorganic components were determined by Mett's method.

We used a mixed food (bread and meat) or bread alone in varying amounts as the food stimulus. Stimultaneously with the feeding, or 1, 2, or 3 hours afterwards, 200, 250, 400 or 500 ml of water 37-38° in temperature were introduced into a rubber balloon previously installed in the stomach.

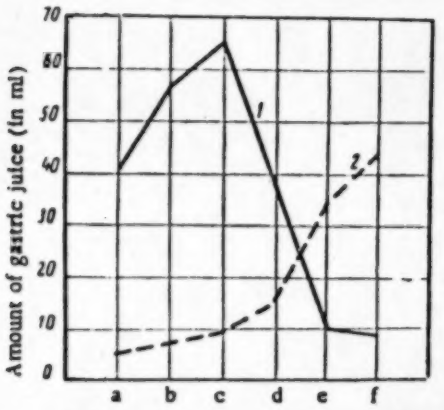
We used an 0.25% solution of hydrochloric acid and a meat-sugar powder as unconditioned stimuli to stimulate saliva secretion. The conditioned stimuli we used were a bell and a spray (sprinkling the oral cavity with distilled water).

The oral cavity was sprayed or the meat-sugar powder was offered every 15 minutes during the experiments, but the first sprinkling or the first offer of the meat-sugar powder was not made until 30 minutes after the animal had been fed.

EXPERIMENTAL RESULTS

The experiments showed that as the amount of food intake increased, the latent period of secretion gradually increased from 5 to 12 minutes and that the latent period of secretion increased 3-8 times, i.e., up to 44 minutes, with the fixed amounts of food. As the amount of food was increased, the amount of juice secreted increased accordingly, but later, with further increases in the amount of food, the amount of juice decreased. The more the amount of food, the less the amount of juice secreted. The free and total acidity of the gastric juice decreased accordingly (see Figure).

Therefore, a greater secretory effect was observed from a one-time feeding of a smaller amount of food than with a larger amount of food.



Change in gastric secretion (1) and latent period of juice secretion (2) with consumption of different amounts of food.
Amount of food: a) 100 g of bread and 50 g of meat; b) 200 g of bread and 100 g of meat; c) 200 g of bread and 200 g of meat; d) 400 g of bread and 200 g of meat; e) 700 g of bread and 200 g of meat; f) 700 g of bread and 300 g of meat.

To prove this fact, the dog was given once a comparatively large amount of food (200 g of meat and 400 g of bread), to which 40.7 ml of juice was secreted in 5 hours. If, however, the dog was given the same amount of food divided into four hourly feedings (50 g of meat and 100 g of bread per hour), more juice was secreted – 67.5 ml, and high tension of the gastric walls was maintained for the whole feeding period (Table 1).

When longer observations (10 hours) were conducted, it appeared that a greater quantity of juice was secreted during the last few hours to the larger amount of food, while, to a comparatively lesser amount of food, the amount of juice secreted was accordingly less.

The same rule was observed in the dogs with the Klementsevich-Heidenhain stomach.

Therefore, different amounts of food had different effects on the gastric glands. First of all, one could observe a uniform secretory effect, secondly, one could obtain a greater effect with a lesser amount of food and a lesser effect with a greater amount of food, and finally, one could obtain complete inhibition of secretion to a large amount of food at a fixed time interval. From the experiments conducted, we concluded that inhibition of gastric juice secretion occurs as a reflex to gastric mechanoreceptor stimulation.

In order to test our proposition, we examined the effect of mechanical stomach wall stimulation on gastric secretion by supplementing the feeding with the introduction of 250 ml water into the balloon installed in the stomach. Gastric secretion became intensified due to the use of comparatively weak stimulation at the same time as the food stimulus. Average data obtained from a dog with a Pavlov stomach is given in Table 2.

In the next experimental series, a comparatively stronger mechanical stimulation of the stomach walls (introduction of 400 ml of water into the balloon) was given simultaneously with the food (300 g of bread). Gastric secretion was considerably inhibited under these conditions; the latent period increased from 12 to 55 minutes, and the total amount of juice decreased. There was a strongly-expressed inhibition in these experiments at first, later replaced by an intensification of secretion. This phenomenon must be caused by the adaptation of the stomach walls to prolonged mechanical stimulation. In order to prevent this adaptation, water was introduced into the balloon and emptied out again every 10-15 mins. In this way, we achieved a more

pronounced, general inhibitory effect than was possible with one-time mechanical stimulation.

TABLE 1

Gastric Juice Secretion with One-Time and Frequent Feeding

Experimental Conditions	Latent period (in min)	Hours	Amount of juice (in ml)	Free HCl (in ml .1N NaOH)	Total acidity (in ml .1N NaOH)
One-time feeding (200 g of meat and 400 g of bread)	9	1	10.8	124	174
		2	9.1	107	155
		3	7.6	128	175
		4	5.5	130	181
		5	7.7	134	183
		Total	40.7	122	172
Four-time feeding (50 g of meat and 100 g of bread each time)	5	1	14.9	130	183
		2	14.9	130	185
		3	14.9	131	185
		4	11.1	129	184
		5	11.7	129	183
		Total	67.5	129	183

TABLE 2

Gastric Juice Secretion on Feeding With 300 g Bread

Experimental Conditions	Latent period (in ml)	Hours	Amount of juice (in ml)	Free HCl (in ml .1N NaOH)	Total acidity (in ml .1N NaOH)
300 g bread	11 5	1	9.3	79	121
		2	4.7	71	114
		3	4.55	54	99
		4	3.45	38	87
		5	2.9	33	87
		Total	24.9	65	111
300 g bread + filling of balloon with 250 ml water	11 0	1	6.8	78	125
		2	8.3	104	147
		3	7.65	106	152
		4	4.2	76	122
		5	4.6	60	100
		Total	31.55	82	131

Mechanical stimulation also inhibited gastric secretion in the dogs with the Klemensevich-Heidenhain stomach.

In order to confirm the fact that impulsation from the gastric mechanoreceptors is of definite importance to the development of inhibition, the mucosa of the greater stomach was sprayed with a 0.25% solution of

novocaine before the mechanical stimulation. We found that, while 19.7 ml of juice had been secreted after 5 hours (latent period of secretion 55 minutes) with the consumption of 300 g of bread and the introduction of 400 ml of water into the balloon in the stomach, then, under the same conditions, after the gastric mucosa had been sprayed with the novocaine solution, 32.5 ml of juice was secreted (latent period of secretion 8 minutes).

Consequently, these experiments prove that reflex inhibition of gastric secretion occurs with strong stimulation of the gastric mechanoreceptors, as with the consumption of a large amount of food.

Examination of conditioned and unconditioned reflex saliva secretion under conditions of both large and small amounts of food consumption showed that both conditioned and unconditioned reflex saliva secretion is sharply inhibited when the dog has consumed a large amount of food (Table 3).

TABLE 3

Amount of Saliva in ml of Conditioned-Unconditioned and Conditioned Stimuli with the Consumption of Various Amounts of Bread

Amount of bread (in g)	Bell + acid	Bell + acid	Bell + acid	Bell	Bell + acid	Bell	Bell + acid
Empty stomach	0.9	2.1	1.8	0.7	1.5	0.6	1.4
200	1.0	2.2	1.9	0.3	1.4	0.3	1.25
400	0.6	1.2	1.25	0.1	0.5	0.1	1.0
600	0.3	0.7	0.5	0	0.3	0	0.5

Note: The saliva was collected for 2 minutes

We observed the same pattern in salivasecretion to the meat-sugar powder as occurred to the irrigation of the oral cavity with a 0.25% solution of hydrochloric acid.

In some experiments, we also examined the effect of teasing on gastric secretion with either an empty stomach or with the balloon in the stomach filled with 500 ml of water. When the balloon was in the stomach, there was almost no gastric juice secreted.

Therefore, the data presented indicate that the more food consumed and the stronger the stimulation (chiefly of the mechanoreceptors) of the stomach walls, the weaker the conditioned and unconditioned reflex secretion of saliva and gastric juice, and the weaker the food reaction. When a very large amount of food is fed, the food reaction changes into a defense reaction – the animal turns away from the food, and saliva and gastric juice secretion is sharply inhibited.

All the facts given above must be considered due to the development of inhibition in the central nervous system caused by strong stimulation of the gastric mechanoreceptors.

SUMMARY

Experiments on dogs showed that single abundant feeding produces less secretion than if the same quantity of food is subdivided into numerous feedings. Inhibition of stomach secretion is reflexive and is connected with the stimulation of mechanoceptors of the stomach. Both conditioned and unconditioned secretions of salivary glands are inhibited.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

ERYTHROPOIESIS IN RADIATION DISEASE COMBINED WITH BURN*

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(Received October 10, 1956. Presented by Prof. V. V. Parin, Active member of the AMS USSR

Both burns and acute radiation sickness cause changes in the peripheral blood. However, the number of erythrocytes in the blood during these influences depends upon erythropoiesis, hemolysis, vascular reactions and, finally, upon bleeding. The reticulocytes, in their turn, do not always exactly reflect the functional condition of the bone marrow [1, 3]. The rate of erythropoiesis in the bone marrow and the rate of erythrocyte entry into the blood can best be determined by the degree of radioactive iron inclusion in the erythrocytes. A series of authors [5, 4, 6, 2] have studied erythropoiesis during radiation sickness with the aid of radioactive iron.

In view of the lack of material in the literature on erythropoiesis during combined affections, we decided to study this question using the labeled atoms method.

EXPERIMENTAL METHODS

One hundred and twenty rats (in 4 groups) were used in the experiments. The animals of the first group were given a one-time general radiation dose of 500 r at a rate of 21 r per minute to the surface in conditions of stable intensity at 180 kv, 10 ma, filter – 0.5 mm Cu + 1 mm Al and with a focusing distance from the skin of 60 cm.

In another group of animals, burns were produced by submerging the lower extremities and the distal part of the body (to the iliac crest) for one second in water 95-100° in temperature. The animals of the third group were subjected to the practically simultaneous action of both factors.

The fourth group contained the control animals.

Ferric chloride, labeled Fe^{59}, with an activity equal to 5 microcuries, in a dose of 1 mg in 0.5 ml of a physiological solution was injected subcutaneously into the rats at different intervals after the injury. Twenty-four hours later, the rats were decapitated. The erythrocytes were separated from the plasma and carefully rinsed; the erythrocyte suspension, in amounts of 0.05 ml, was applied to two targets, and the radioactivity of the erythrocytes was then determined with a B apparatus with an end-window counter. Identical conditions of radioactive iron determination made it possible to find any erythropoietic change in the injured animals as compared with the control animals.

EXPERIMENTAL RESULTS

With one radiation treatment, erythropoiesis was sharply inhibited on the very first day (see Table) and was practically nonexistent between the 2nd and 5th days after the radiation, since, as experiments we conducted earlier have shown[2], the inclusion of iron in the peripheral blood erythrocytes can cause slight erythrocyte radioactivity.

* Presented at the IV Azerbaidzhan conference of Roentgenologists and Radiologists, May 19, 1956.

Radioactive Iron Inclusion in Erythrocytes of Injured Rats Expressed in Percent of Eruthrocyte Radioactivity in Healthy Animals

Type of Injury	Days after injury							
	0–1	1–2	3–4	5–6	7–8	9–10	11–12	15–16
General irradiation in dose of 500 r	32	10	15	28	56	87	104	129
Boiling water burn	125	160	121	84	70	52	73	86
General irradiation+ burn	28	18	10	12	16	23	27	35

Later, erythrocyte production increased, and, after two weeks, it had returned to normal.

With the burn, on the other hand, erythropoiesis was considerably intensified during the first few days following the burn: the iron inclusion in the erythrocytes began to decrease after the 5th day, reaching 52% of normal by the 10th day. Erythrocyte formation had still not reached the original level on the 16th day. With the burn, therefore, the primary changes of the blood were caused by true intensification of erythropoiesis as well as by redistribution of the blood and plasma loss. The subsequent inhibition of the bone marrow function is evidently connected with the inflammatory complications. We have established that the erythropoietic activity of the bone marrow is inhibited in animals with inflammatory processes, artificially induced by an injection of a culture of various microorganisms.

With the combined injury, erythropoiesis and the exodus of erythrocytes from the bone marrow ceased almost completely during the first 10 days after the injury.

Fifteen days after the radiation, erythrocyte radioactivity was still three times less that of the control.

In each series of killed animals, iron inclusion in the erythrocytes did not vary much in the individual rats during the first few days, but subsequently, there were considerable differences. For example, erythrocyte radioactivity in three animals which had been subjected to the combined injury and killed 24 hours later was 22-32%, the average being 28%, while after 15 days, in three other rats, erythrocyte radioactivity fluctuated from 14 to 71%, with an average of 35%; the other 7 rats which had been kept for this interval died.

One must mention that the unit activity of the erythrocytes reflected erythropoiesis well the first few days after the radiation; the very slight increase of radioactivity with progressing anemia did not yet indicate the restoration of hemopoiesis, since the activity of a definite amount of erythrocytes did not depend on the amount of erythrocytes in the peripheral blood.

With the same number of radioactive erythrocytes released by the bone-marrow, erythrocyte unit acitivity would be more rather than less that of the erythrocytes in the peripheral blood.

Nevertheless, with one radiation, erythrocyte radioactivity had increased by 29% after 15 days and the number of erythrocytes had decreased by 20%, which indicates that restoration of erythropoiesis was complete at this time.

Besides these experiments, the serum iron was determined in 24 rats on the 2nd and 6th days after each type of injury. The easily-released iron increased most with the combined factors; with the burn, the increase was most expressed on the 2nd day after the injury, but, with the radiation sickness, on the 6th day.

Therefore, with a combination of burn and radiation sickness, there is maximum erythropoiesis inhibition and hemolysis intensification, which leads to the rapid development of expressed anemia.

SUMMARY

Experiments with radioactive iron have demonstrated that in rats subjected to x-ray irradiation (500 r) erythropoiesis is for the first ten days inhibited, and restored at the end of the first week. In rats with burns of

the distal parts, caused by boiling water, erythropoiesis is enhanced during the first days and then with the development of inflammatory complications, is inhibited, not being restored even at the end of two weeks. In animals subjected to both irradiation and burns, release of erythrocytes ceases almost completely and in surviving ones restoration of erythropoiesis proceeds very slowly.

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SOME PECULIARITIES IN THE REGULATION OF THE RATE OF HEART CONTRACTIONS IN CORONARY INSUFFICIENCY

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It is known how quickly and responsively the rate of the heart contractions changes due to different environmental changes. Emotional influences and the effects of stimuli causing a superficial reaction lead to this or that change in the heart rhythm. Pulse retardation or acceleration is one of the ways in which the body responds to the stimulus in the realization of many reflex acts.

Pathological changes occurring in the organ due to a pathologic process alter both the condition of the organ as such and its reflex responses. A series of authors studying the vegetative cardiac reflexes noted certain peculiarities in their course in patients with diseases of the myocardium. Wenckebach [6] used the Chermak's reflex for diagnosis, as he considered that acute expression of this reflex was typical of serious heart patients. A. Weil [5] proposed that this intensified reaction was connected with pathologic change in the coronary vessels. L. Braun and B. Samet [4] discovered in an experiment that the effect which stimulating the peripheral termination of the vagus nerve has on the heart is intensified when the coronary arteries are impaired.

M. E. Mandelshtam and others [2, 3], studying vegetative cardiac reflexes in patients with inflammatory and degenerative-infiltrative processes in the heart, found that the carotid sinus reflex was often acutely expressed, whereas, in patients with only inflammatory signs in the heart, the reflex was expressed to the same degree as in healthy people.

V. M. Kogan [1], in a psychological study of patients with coronary insufficiency, found that their heart rhythm accelerated less in response to emotional stimulation.

Our study was based on this observation.

EXPERIMENTAL METHODS

The purpose of our work was to study the changes in the rate of the heart contractions in coronary insufficiency patients in response to stimuli normally causing both acceleration and retardation of the heart contractions. The stimuli we used were emotional stimulation, nitroglycerin , pressure on the eyeballs (Aschner's reflex) and pressure in the region of the carotid sinus (carotid sinus reflex). We recorded the pulse continuously during these various influences and also the electric reflex and the vascular reactions (by plethysmography); electrocardiograms were taken in the second lead (Fig. 1).

The emotional stimuli we used were as follows: the patient was questioned briefly about the pains in his heart and the reasons first causing them and a series of problems were successively suggested to him, some of which encouraged the patient as he could easily cope with them, and others which perplexed him, causing the patient either to refuse to solve them or, on the other hand, to become agitated and try to solve them at all costs. The reactions in response to the emotional stimuli were examined in colaboration with V. M. Kogan.

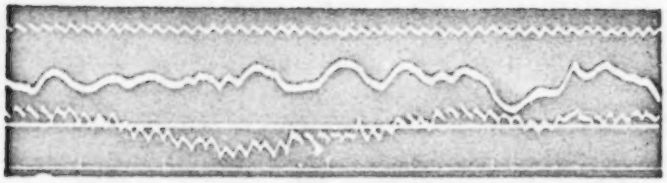

Fig. 1. Simultaneous recording of pulse, electric-skin reflex and plethysmogram (from finger).
Curves signify, from top to bottom: pulse, electric-skin reflex, plethysmogram, zero line, time indication (in 5 second marks).

As the material was developed, the heart contractions were counted every 15 seconds throughout the investigation. The records were analyzed by computing the height and frequency of the waves recorded by the electric-skin reflex, the background oscillation and the degree to which the vascular reactions were expressed in the plethysmograms. The intensity of the electric-skin reflex and the vascular reactions was expressed in conditional marks (according to the triple-mark system).

We observed a total of 112 persons: 106 patients with pains in the region of the heart and 6 healthy people.

The patients were divided into three groups according to the classification of L. I. Fogelson, which has been accepted by TsIETIN: the first contained those persons with pains of coronary origin in the heart (28 persons), the second (control), those with pains of noncoronary origin in the heart (30 persons), and the third, those with pains of mixed origin in the heart (48 persons). In the last group, coronary insufficiency was the main source of the pain in 31 persons (group 3-a), while pains of a noncoronary origin predominated in 17 persons of this group (group 3-b).

The average frequency of the heart contractions, which was first examined daily in the clinic and then under the experimental conditions before our experiments, was about the same in all of the groups.

EXPERIMENTAL RESULTS

At certain moments during the emotional stimulation, the heart rhythm changed considerably by becoming either faster or slower than the original pulse rate.

The maximal acceleration of the heart contractions as compared with the original rate was much smaller in the patients of the 1st group – a total of 6.5 beats, while the total was 19.8 beats in the patients of the 2nd group (Fig. 2). The acceleration of the heart rhythm in the 3rd group was intermediate as compared with the first two groups and consisted of 14 beats.

The maximal acceleration in the healthy people was 17 beats.

The average maximal retardation was the same in all of the experimental groups (Fig. 2), but it occurred more often in some patients of the 1st group. There was no specificity observed for any particular group of patients in the manifestation of other vegetative reactions (electric-skin reflex and vascular reactions); there was approximately the same number of acute, moderate and weak reactions observed in each group of patients.

Therefore, although the reactions in response to the emotional stimuli were identical as far as the other vegetative functions were concerned, the reaction of heart rhythm change was electively diminished in the coronary insufficiency patients, as was expressed in the lesser acceleration of the heart contractions observed in these patients.

The data described confirm similar phenomena observed earlier by V. M. Kogan.

In the remaining part of the work, we analyzed the effects of other stimuli on the heart rhythm.

When nitroglycerin was used as the stimulus, the heart contractions accelerated one minute after its administration in all of the patients, reaching the maximum acceleration by the third minute (16-44 beats). The blood pressure (maximal, average and minimal), which was measured by arterial oscillograph 3 and 5 minutes after the administration of nitroglycerin to the patients, did not change in most cases.

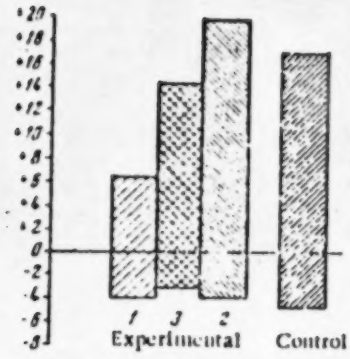

Fig. 2. Maximal acceleration and retardation of pulse as compared with original rate.
1, 2, 3) different groups of patients.

In the patients of the 1st group, the acceleration of the heart contractions observed after the administration of nitroglycerin (an average of 19 beats) was somewhat less than in the control group (an average of 24 beats).

Aschner's reflex caused the heart contractions to become 4-8 beats slower. No great differences as to the extent to which the heart rhythm was retarded were observed between the patients with pains of coronary origin and those with pains of noncoronary origin. In the patients of the 1st group, however, a retardation approximating 8 beats was more frequently observed than in the patients of the 2nd group, in which the retardation was usually 4 beats. Therefore, one could observe a greater tendency to retardation of the heart contractions in the patients with a coronary insufficiency.

With pressure on the carotid sinus region, the heart contractions slowed in 98% of the patients of the first group, in most cases, by 12 beats. The heart contractions were only slowed in 28% of the patients of the 2nd group, and then, only by 4-8 beats. The heart rhythm was either not retarded at all in the healthy persons, as occurred in the majority of cases, or by a total of only 4 beats.

Consequently, the carotid sinus reflex (which slows the heart rhythm) was somewhat stronger in the patients with pain of coronary origin than in the patients of the other groups.

The electrocardiograms, which were taken during the action of both the emotional stimuli and the other stimuli, did not show any changes indicating that dynamic disturbances of coronary circulation had developed in the course of our study.

Therefore, in comparison with the patients with pain of non-coronary origin and with the healthy people, a diminished ability to alter the rhythm of the heart contractions was observed in the patients noncoronary ailments during the action of stimuli causing heart rhythm acceleration (emotional stimuli, nitroglycerin).

There was a more pronounced reaction of heart rhythm retardation observed in these patients with the action of stimuli causing retardation of the heart contractions.

Therefore, peculiarities which were evidently of a compensatory-defense nature could be found in the reflex cardiac activity changes of the coronary insufficiency patients. Due to these peculiarities, the heart rhythm with various external and internal stimuli was slower than in healthy people. The lack of significant heart rhythm acceleration can be regarded as a favorable phenomenon, as acceleration would have made increased demands on an imperfect coronary system and thus cause acute coronary circulation insufficiency.

The lesser acceleration of heart rhythm in the coronary insufficiency patients cannot be explained by a reduction of general reactivity. The absence of any specific changes in the reactions of the other vegetative functions indicates the specificity of extracardial heart rhythm regulation in coronary insufficiency patients.

One can propose either that this is due to the altered reactivity of the heart itself caused by pathological change, or, which we believe is more probable, that it is due to a reconstruction of the reflex mechanisms regulating the heart rhythm, causing a relative predominance of inhibitory effects.

SUMMARY

The rate of heart contractions, electric-skin and vascular reactions to different agents exciting and inhibiting the rate of heart contractions have been studied in patients with coronary insufficiency. A diminished ability to alter the rate of the heart rhythm has been disclosed in such patients. Since no specific peculiarities have been demonstrated in the reactions of other negatative functions, it is assumed that in coronary patients changes of extracardial regulation of heart rhythm are specific.

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CAROTID SINUS REFLEXES WITH FOREIGN BLOOD STIMULATION OF THE SPLEEN INTEROCEPTORS

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The pathogenesis of circulatory and respiratory disturbances due to blood transfusion shock is not yet clear.

According to the data of A. N. Gordienko [1], the carotid sinus circulatory reflexes are profoundly changed in dogs due to blood transfusion shock caused by an intravenous infusion of heterogeneous blood. The indirect action of foreign blood on the receptors of an isolated carotid sinus results a similar picture [2]. In the opinion of the authors cited, this phenomenon indicates that the function of the peripheral neuroreceptive apparatus is profoundly disturbed during shock, and these disturbances perhaps play a principal role in the pathogenesis of hemodynamic changes.

Accepting the premise of contemporary physiologists that the afferent impulsation emanating from the receptors of the cardiovascular system plays an important part in the maintenance of the tonicity and excitability of the centers regulating circulation and regulation, we decided to investigate the mechanism of the phenomenon mentioned.

EXPERIMENTAL METHODS

The experiments were done on dogs, in which the spleen had been humorally isolated according to V. N. Chernigovsky [4] under ether or hexenal* anesthesia, after the anesthesia had passed.

0.2 ml of a solution of lobeline hydrochloride or acetylcholine (1:25,000), or of a 0.2% solution of sodium sulfide, was introduced into the central part of the superior thyroid artery in order to stimulate the chemoreceptors of the carotid zone.

The intensity of the carotid sinus circulatory and respiratory reflexes was determined from the rise in arterial pressure and the change in the rhythm and depth of the respiratory movements, which were recorded in the course of the experiment.

The reflexes were examined at the following intervals: before laparotomy and humoral isolation of the spleen, after splenic isolation, on a background of splenic perfusion with citrated rabbit's blood and 15, 30 and 45 minutes after perfusion. Perfusion of the humorally isolated spleen with the citrated rabbits' blood lasted 5 or 10 minutes.

EXPERIMENTAL RESULTS AND EVALUATION OF RESULTS

It has been established that the operation of opening the celiac cavity in order to humorally isolate the spleen affects the course of the carotid reflexes. The carotid reflexes caused by lobeline were the most changeable, and the reflexes caused by sodium sulfide were the most resistant.

As a rule, the reflexes decreased in magnitude, and the presence of a depressor phase, replacing the pressor beginning of the reaction, was frequently observed.

* Russian trade name.

Only in 3 out of 19 cases did the carotid reflex to lobeline increase, and in four cases, after the operation, the reflexes to sodium sulfide increased.

Opening the celiac cavity and manipulating its organs evidently changes the functional condition of the nervous system. Consequently, reflexes caused by the stimulation of carotid sinus chemoreceptors are changed.

The carotid reflexes did not diminish during the splenic perfusion with foreign blood, which led to a pressor-depressor reaction, but, conversely, showed a definite tendency to increase (Fig. 1). This tendency was strongest in the reflexes caused by lobeline and sodium sulfide. In the first case, the hemodynamic component of the reaction was most intensified, in the second, the respiratory component (Fig. 2 and 3).

A slight decrease in the reflex reaction to acetylcholine occurred in 12 out of 19 experiments; a biphasic reaction was observed in 3 experiments, and an inverted circulatory reaction even occurred in 2 experiments, expressed by arterial pressure depression. The respiratory component of the carotid reflex, however, was almost unchanged.

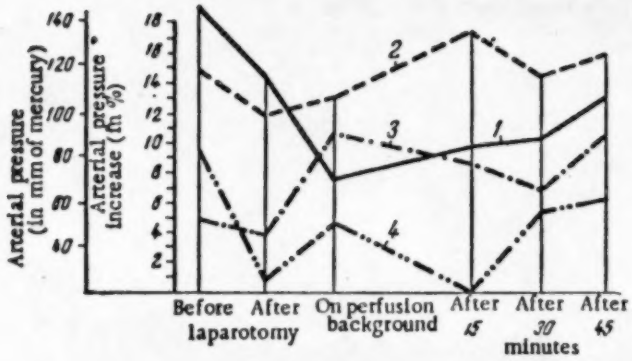

Fig. 1. Changes in carotid circulatory reflexes with foreign blood perfusion of the spleen.
1) arterial pressure; 2) reflexes to lobeline; 3) reflexes to sodium sulfide; 4) reflexes to acetylcholine.

In 3 experiments in which the carotid sinus reflex to acetylcholine was pronounced from the very beginning, foreign blood stimulation of the spleen interoceptors caused it to diminish.

The presence of an expressed reaction to lobeline during the hypotonia caused by the pathologic stimulation of the spleen interoceptors with foreign blood indicated the great stability of this reaction and the possibility that it has a compensatory-defense significance. The compensatory-defense character of this reaction is confirmed by the fact that, in the experiments in which the carotid reflexes to lobeline increased 15 and 30 minutes after the heterogeneous blood irrigation, the arterial pressure, which had dropped due to this irrigation, was completely restored after even shorter intervals. When the reflex reaction to lobeline decreased due to excessive stimulation of the spleen receptors with the rabbit's blood, restoration of arterial pressure proceeded much more slowly. Analyzing the dynamics of the carotid sinus reflexes to sodium sulfide disclosed an analogous picture.

We could not establish a strict relation between the arterial pressure changes in the dogs in which the spleen receptors were stimulated with heterogeneous blood and the character or intensity of the carotid sinus reflexes caused by acetylcholine. Moreover, in spite of the presence of inverted carotid sinus reflexes to acetylcholine, in 2 experiments, one could observed a steady restoration of arterial pressure after foreign blood perfusion of the spleen. This makes it possible to propose that the nervous mechanisms producing the reaction from the carotid chemoreceptors to acetylcholine do not play an essential part in the restoration of arterial pressure.

Analyzing the results obtained from studying the reflex reactions of the reflexogenic carotid sinus zone to various chemical stimuli permits one important point to be established.

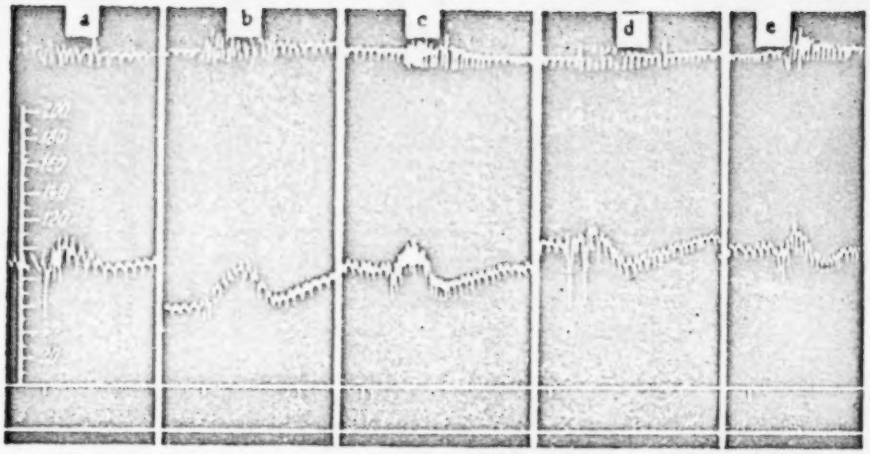

Fig. 2. Reaction from receptors of carotid sinus zone to lobeline during the action of foreign blood on the spleen interoceptors.
a) carotid sinus reflex on circulation and respiration before foreign blood perfusion of spleen (after spleen isolation); b) the same on a background of foreign blood perfusion; c,d,e) the same 15,30 and 45 minutes after perfusion. The curves from top to bottom signify: respiration, arterial pressure, zero line and indication of stimulation, indication of time (in 1 second marks).

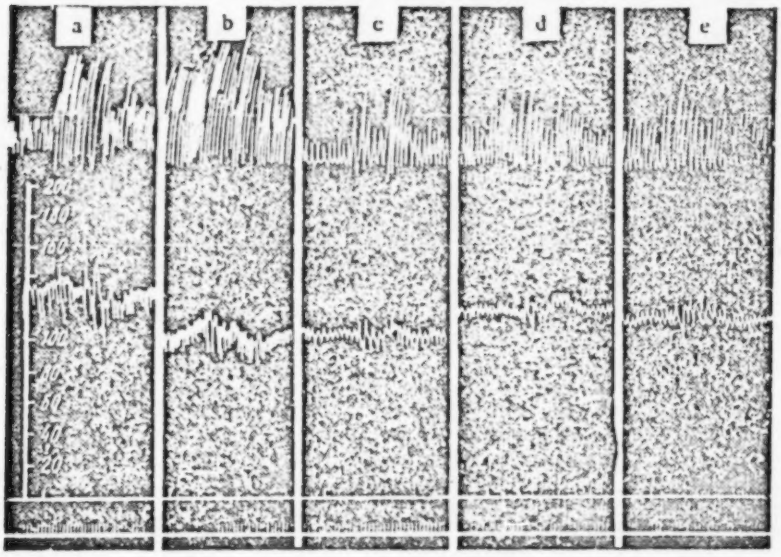

Fig. 3. Reaction from the receptors of the carotid sinus zone to sodium sulfide with the action of foreign blood on the spleen interoceptors.

Stimulating the splenic receptor field with heterogeneous blood under conditions of splenic isolation from general circulation can change the condition of the reflexes arising from another receptor field, that of the carotid reflexogenic zone, far from the direct action of the blood.

We proposed that the mechanism of such a change is reflex and is connected with changes in the nervous processes in the carotid sinus reflex centers. If our proposition is valid, then, no matter to which reflexogenic zone the action of the blood is primarily directed, the effect should be the same.

In order to prove this proposition, we conducted supplementary experiments. The receptors of an isolated carotid sinus were subjected to the action of heterogeneous blood, and the reflex reactions from the intestinal interoceptors to the introduction of the same chemical stimuli, with the exception of sodium sulfide, were examined.

The carotid sinus was isolated from general circulation by the method described by A. N. Gordienko and V. L. Volkotrub [2], and the section of the small intestine was isolated according to V. N. Chernigovsky's method [4].

Solutions of the chemical substances, in the same concentrations as before, were introduced into a perfusion current irrigating the isolated intestine, in amounts of 1.2 ml.

The reflex reactions to these stimuli were examined before carotid sinus perfusion with foreign blood, on a perfusion background and 15 and 30 minutes after the perfusion.

Two particularly interesting facts resulted from the analysis of the results obtained in these experiments: during carotid sinus perfusion with heterogeneous blood, the reflex reactions from the intestinal receptors to the chemical stimuli greatly diminished; during the period in which these reactions were restored to their original magnitude, a phase of increase occurred at the time when foreign blood perfusion of the carotid zone was halted.

One can propose that the heterogeneous blood stimulation of the carotid zone chemoreceptors causes a condition similar to parabiosis in the central nervous system. When this stimulation ceased, the functional condition of the central nervous system (or more exactly, the central links in the arc of the studied interoceptive reflexes) is restored.

The phase of increased reflex reactions present during the restoration period can be considered as a manifestation of the paradoxical stage of parabiosis in the course of slow involution.

The experiments conducted, then, confirm the proposition that change is possible in the reflex reactions to chemical stimuli from receptor fields far removed from the place of the pathogenic stimulus's direct action.

The change in the magnitude of the reflexes originating from receptor zones not in direct contact with the pathologic stimulus possibly have either a defense-adaptive or a pathologic significance to the body, depending on the conditions of development.

SUMMARY

It was established in experiments on dogs that when isolated vascular zones (carotid sinus and spleen) were perfused with heterogeneous blood, reflex changes in the reactions to chemical stimuli occurred. This is true of stimuli that act on receptor fields (intestinal receptors, carotid zone chemoreceptors) far removed from the place of direct action of the stimuli.

There is no direct relationship between the degree of disturbance of circulatory reflexes of the zone of carotid sinus (was revealed in perfusion of isolated spleen by heterogeneous blood) and the changes in the blood pressure which are found at that time.

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THE EFFECT OF VITAMIN B_1 ON DIGESTIVE GLAND SECRETION

COMMUNICATION I. GASTRIC GLAND SECRETION IN DOGS WITH EXPERIMENTAL VITAMIN B_1 DEFICIENCY

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(Received March 31, 1955. Presented by Prof. V. N. Chernigovsky, Active Member of the AMS, USSR)

One of the most important questions in research concerning the mechanisms regulating digestive tract activity and their normal and pathologic states is the role played by vitamins in digestive gland activity. In spite of the numerous works on this subject, the exact effects of the various vitamins on the digestive glands has not yet been definitely established.

Many of our colleagues have studied the effect of various vitamins on the activity of gastrointestinal tract organs and tissues (N. V. Asmayan, A. V. Gubar, O. F. Sharovatova, S. I. Filippovich, Yu. N. Uspensky, N. G. Shchepkin and others). For the past few years, we have been making a study of experimental B_1-avitaminosis.

In research prior to this, B_1-avitaminosis with typical clinical symptoms and metabolism disturbance has been produced in dogs fed various diets (synthetic and autoclave-processed). It has also been established that the pathologic body condition caused by B_1-avitaminosis can be reversed by timely treatment with vitamin B_1. The experimental model obtained made it possible to proceed with the study of digestive gland (salivary and gastric) secretion in dogs with B_1-avitaminosis.

We found a series of authors in the literature (Tsitovich and Levinson, Levinson, Bikkel, Never and others) who came to conflicting conclusions after studying gastric secretion in dogs with polyavitaminoses and an isolated miniature stomach. Some authors found no changes of gastric secretion in B_1-avitaminosis, while others observed great changes in the secretory function.

Among the experimental works directly associated with our study, the unpublished work of N. A. Izmailova, conducted in the laboratory of Prof. M. P. Brestkin, should be mentioned. Her experiment showed that the reflex phase of gastric secretion diminished with B_1-avitaminosis and that the humoral phase of gastric secretion at first, grows, and then, during the last days before the death of the animal, diminishes greatly.

In our work, we studied the dynamics of gastric gland secretion in dogs during the development of B_1-avitaminosis and during the subsequent vitamin therapy.

EXPERIMENTAL METHODS

Gastric secretion was studied in dogs with an isolated miniature stomach.

The weight, diuresis, concentration of pyruvic acid in the urine, temperature, pulse and number of respirations were systematically determined, and the amount of food and water consumed daily was ascertained.

After a general background and a background of gastric secretion to the stimuli employed had been established, the animal was put on one of the experimental diets. During the development of experimental B_1-avitaminosis in the animals, we systematically recorded the changes in the secretion indices of the gastric glands and compared them with the clinical symptoms. The method and general and dietary conditions used to obtain experimental B_1-

avitaminosis in the dogs we have already described in detail in a previous article.

We used 100 g of autoclave-processed meat or 100 ml of 10% alcohol as stimuli (the alcohol was administered to the dogs rectally). The experiments were done daily; the amount of secretion, acidity and the digestive power of the juice were determined according to Mett.

The results given in this work were obtained from 4 dogs, 3 of which (Boy, Beetle and Topper) had a stomach isolated according to Brestkin-Savich, while the other (Daisy) had a Pavlov's miniature stomach and a gastric fistula. Two of the dogs (Boy and Beetle) were fed a casein saccharide diet, and the other two (Daisy and Topper) were fed an autoclave-processed diet. Over 500 experiments were conducted.

EXPERIMENTAL RESULTS

The first signs of avitaminosis sickness (diminished appetite, periodic refusal to eat and loss of weight) appeared in the experimental animals on the 15th-20th day after the beginning of the experimental diet. Deviations from normal gastric gland secretion appeared at the same time and intensified as the avitaminosis developed.

Experimental results are given in Figs. 1 and 2.

A slight qualitative change in secretion was observed in the animals during the first month of the vitamin deficient diet. Later, secretion became more profoundly disturbed; secretion fluctuated greatly to both stimuli, and gastric juice was secreted on an empty stomach.

In the experiments with the 100 g of autoclave-processed meat (Fig. 1), the amount of gastric juice was observed to fluctuate, the amounts primarily increasing, more and more as avitaminosis developed.

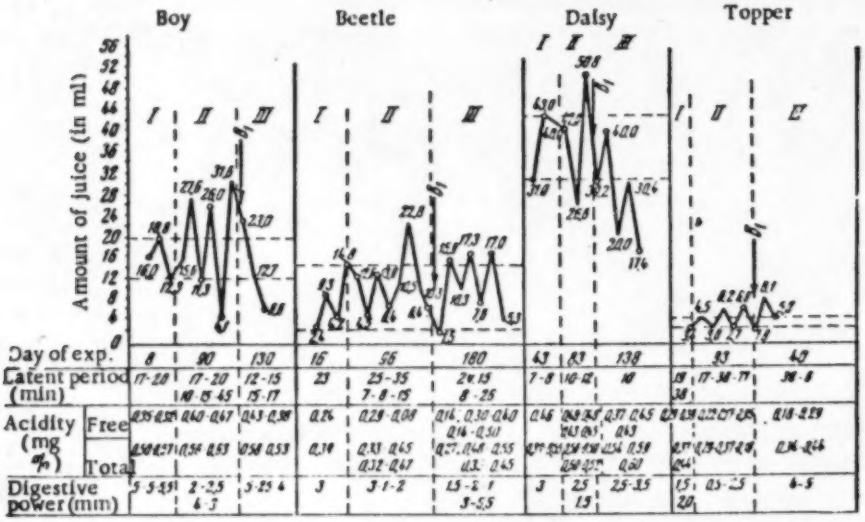

Fig. 1. Gastric secretion to 100 g of autoclave-processed meat. The fluctuation limits of gastric juice secretion in milliliters to the given stimulus are marked by the two horizontal dotted lines. Along the abscissa - duration of the period in days. Along the ordinate - amount of juice in milliliters (each number represents the arithmetical mean of fluctuations from several experiments in the given period). Numbers near dots on graph - amount of secretion in one experiment.
The periods are divided by the vertical dotted lines: I) background, II) development of avitaminosis, III) restoration.
Arrows signify the critical day of the disease and the first vitamin B_1 injection.

In the dog Boy, as Figure 1 shows, the amount of gastric juice, normally 18.8 ml, increased from 27.6 to 31.6 ml. In Beetle, the secretion, normally 14.8 ml, reached 22.8 ml. In Topper, the juice secreted during the development of avitaminosis was twice the normal amount. For example, juice secretion, normally 3.4 ml, increased to 6.2 ml, then sank sharply to 1.8 ml on the critical day of the disease. Secretion in the dog Daisy fluctuated at a level slightly lower than the norm (26.6 ml), with a sharp incline to 51 ml 12 days before the crisis.

Secretion fluctuations were also observed in the experiments with the rectal introduction of 100 ml of 10% alcohol, but the amount of gastric juice secreted was considerably less than normal.

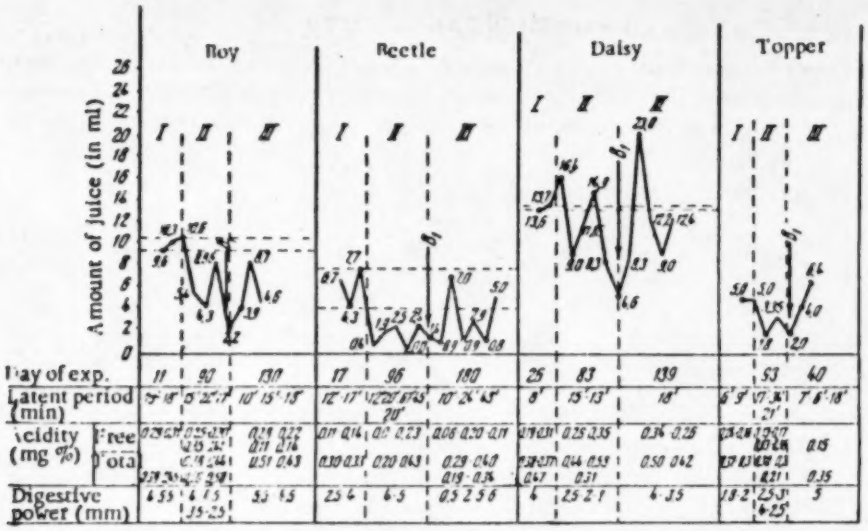

Fig. 2. Gastric secretion to 100 ml of 10% alcohol.
The symbols are the same as in Fig. 1.

Figure 2 shows that in the dog Boy, fluctuations of secretion, normally 9.6-10.6 ml, were observed during the development period of avitaminosis which were 4.3-8.8 ml, with a decrease to 2.2 ml on the critical day of the disease. As the avitaminosis condition became more serious in the dog Beetle, secretion sharply declined, reaching 2.5 and 0.0 ml in the acute stage of the avitaminosis, when the normal secretion was 4.3-7.7 ml. Secretion fluctuations were also lower than the norm in Topper (from 1.8 to 3.3 ml).

As the disease developed in the dog Daisy, sharper, intermittent secretion fluctuations above and below normal limits were observed. Thus, secretion, normally 13 ml, increased to 16 ml, then fell to 9 ml, increased again to 14 ml and then steadily decreased until the critical day of the disease when it was 4.6 ml.

Therefore, the experiments conducted showed that fluctuations occur in gastric secretion (hypersecretion or hyposecretion) to meat and alcohol during the development of avitaminosis.

Besides the changes which occurred in the amount of gastric juice during the development of avitaminosis and the subsequent vitamin therapy, changes were also observed in the latent period of secretion, the acidity and the digestive power of the juice to both stimuli (meat and alcohol).

Figure 1 shows the changes in the latent period, acidity and digestive power according to the periods (extreme limits of fluctuation are given). As the graph shows, the acidity of the gastric juice changes in the same manner as the amount of gastric juice, but not as much.

In some of the dogs, during the acute stage of the disease, we observed a divergence between the degree of acidity and the amount of gastric juice, which seemed to be connected with the changed trophic function of the glands.

During the development period of avitaminosis, the digestive power of the juice decreased, returning to normal, or sometimes to above-normal, only after long treatment with vitamin B_1 (Figs. 1 and 2). For example, the digestive power of the juice to meat in the dog Boy, Normally 5-6 mm according to Mett, had decreased by the first week of the vitamin-deficient diet to 2-2,5 mm, and only returned to normal after a month of treatment. During the development period of avitaminosis, the digestive power of the juice in Beetle fluctuated between 3 and 1 mm (the normal being 3 mm) and remained 2 mm for a month before the crisis, returning to normal only after 2 months of vitamin therapy. In Daisy, the digestive power returned to normal a week after vitamin therapy.

It is evident from the graphs that the latent period during the development of avitaminosis was variable-sometimes being longer, sometimes shorter. In some experiments, however, it became longer to both stimuli. For example, the latent period in the dog Boy, normally 17-20 minutes, became as long as 45 minutes. In Beetle, the latent period, normally 23 minutes, decreased to 7-8 minutes, then lengthened to 2 hours, 15 minutes. In the dog Topper, it lengthened to 77 minutes, i.e., became twice as long as the normal latent period.

Vitamin B_1 was injected intramuscularly (10 mg thiamine hydrochloride on the critical day of the disease when the animal was in a serious condition. Fifteen to twenty minutes after the vitamin B_1 injection, the general condition of the dog improved, vomiting ceased, and the complete refusal to eat was replaced by an extraordinary appetite. Thereafter, the sick dog was injected daily with 5-10 mg of thiamine hydrochloride until his complete recovery.

Vitamin therapy caused gastric secretion to become almost normal. At first, sharp secretion fluctuations were also observed during the treatment; there was still some decrease in secretion to meat and increase in secretion to alcohol in three of the dogs, Daisy, Boy and Beetle.

Therefore, the data obtained show that B_1-avitaminosis causes considerable distrubance in gastric gland secretion and also that there is a definite relation of gastric secretion to the degree to which the pathologic process occurring in the body with the development of B_1-avitaminosis is expressed.

Microscopic examination of the mucous membrane of the stomach in dogs with B_1-avitaminosis (Rudik-Gnutova) showed considerable hyperemia of the vessels of the mucous and submucous layers. The mucosa itself retained its normal structure, while a loss of secretory granules and vacuolization of the protoplasm could only be observed in the chief and cover cells, which could relate to the functional changes connected with the secretion of gastric juice.

The numerous experimental works and the mass of clinical literature treating vitamin B_1's effect on the nervous system show the close relation between thiamine and the function of the nervous system.

Vitamin B_1 is also known to play an extremely important part in body metabolism and in the metabolism of some body tissues – nervous, glandular, etc. Consequently, one can propose that a vitamin B_1 deficiency in the body can cause changes and distrubances in gastric secretion because of the metabolic changes occurring in the glandular tissue, in the nerve plexi of the digestive tract, in the central nervous system and in its highest division – the cerebral hemispheres.

A comparison of the physiological data we obtained with the data from our histological examinations, which showed an absence of organic distrubances in the glandular cells of the gastric mucosa, led us to propose that the distrubance of gastric gland secretion which occurs in B_1-avitaminosis is functional and depends on neural regulation.

Which of the divisions of the nervous system regulating gastric secretion is the most affected by B_1-avitaminosis is a subject for further investigation.

SUMMARY

The quantity of secretion, its acidity and power of digestion was studied in experiments on dogs with isolated Paubu's stomach in condition of B_1 insufficiency. Mett's method was employed. It has been established that vitamin B_1 insufficiency causes pronounced disturbances in digestive glands. The power of digestion of the stomach juice drops from 5-6 cc to 2-2,5 cc. The quantity of stomach juice increases up to 31.6 cc, the normal being 18.8 cc.

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BIOCHEMISTRY AND BIOPHYSICS

THE ROLE OF DYNAMIC CARDIAC NERVES IN THE REGULATION OF MYOCARDIAL TROPHICS

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I. P. Pavlov established the anatomic individuality of nerves which altered the strength of cardiac contractions, and devoted particular attention to the nature of these nerve fibers which he called "dynamic." Analysis of the action of these nerves on the heart led Pavlov to conclude that these were special nerves exerting a trophic effect on heart muscle. At the same time, Pavlov said, "this question may be raised and discussed, but not settled finally" [1].

It would appear that direct proof of trophic influences of dynamic nerves could be obtained only from biochemical examination of the myocardium. Of particular interest, therefore, is one of the recent communications of M. E. Raiskina [2] who showed that the "reinforcing" cardiac nerve increases the process of phosphorus fraction turnover in the myocardium (inorganic phosphorus, adenosinetriphosphoric and creatinephosphoric acids).

In the present work are presented results of investigations of the effect of dynamic cardiac nerves, parasympathetic and sympathetic, on the metabolic process of the myocardium. The following were taken as indices of myocardial metabolism: tissue respiration, content of phosphorus fractions (inorganic phosphorus, adenosinetriphosphate and phosphocreatine) and glycogen content.

METHODS

The experiments were performed on dogs of approximately the same age and weighing 8-13 kg. The dogs were kept under identical conditions and on identical diets; they were kept fasting for 24-30 hours prior to beginning the experiments.

Experiments were carried out at different times of the year. The thorax was opened under hexenal*-morphine anesthesia (artifical respiration), the vagus was followed along its course, and the cardiac branches leaving the inferior cervical sympathetic ganglion were dissected. The nerves were stimulated by induction current obtained from a Zimmerman apparatus with a 2 v accumulator. Stimulation of the nerves was from 1 to 4 minutes in duration. Cardiac activity was recorded on a smoked drum. Since interest centered on the influence of dynamic cardiac nerves on myocardial trophics only, the data of biochemical analysis reported in the present paper are concerned only with experiments in which stimulation of cardiac nerves led to changes in the strength of cardiac contractions (increase or decrease) without changes in cardiac rhythm. After a precisely determined period of time from the beginning of stimulation, a portion of the heart in the region of the left ventricle was removed; part of the tissue was frozen in liquid oxygen, while the remainder was used for determination of tissue respiration.

* Russian trade name.

Respiration was measured in a Warburg apparatus with alkali in the middle reservoir, using heart muscle macerated with phosphate buffer (pH 7.4) at 26°C.

Phosphorus fractions and glycogen were determined on heart muscle frozen in liquid oxygen. The frozen tissue was triturated. The proteins were precipitated with trichloracetic acid in the cold. Inorganic phosphorus was precipitated from protein-free filtrate, in the cold, by an equal volume of magnesial mixture, and estimated by the Fiske-Subbarow method. Phosphocreatine was determined in the filtrate after the precipitation of inorganic phosphorus; in the presence of molybdate in an acid medium, it broke down with the formation of inorganic phosphorus, which was estimated as described above. ATP (adenosinetriphosphate) was isolated as the barium salt from a separate portion of the trichloracetic filtrate, and determined by the amount of inorganic phosphate split off following 7-minute hydrolysis in 1N HCl on a boiling water bath. Inorganic phosphorus was determined simultaneously, but without preliminary hydrolysis in the other portion of the same solution.

Glycogen was isolated from the tissue, hydrolyzed and estimated as glucose by the iodometric method. In total, 50 experiments were performed: 27 with stimulation of the nerves, and 23 controls.

RESULTS

The whole dynamics of biochemical changes associated with a positive inotropic effect is represented graphically in Fig. 1. The diagram is based on the arithmetic mean of data expressed as percentage of control.

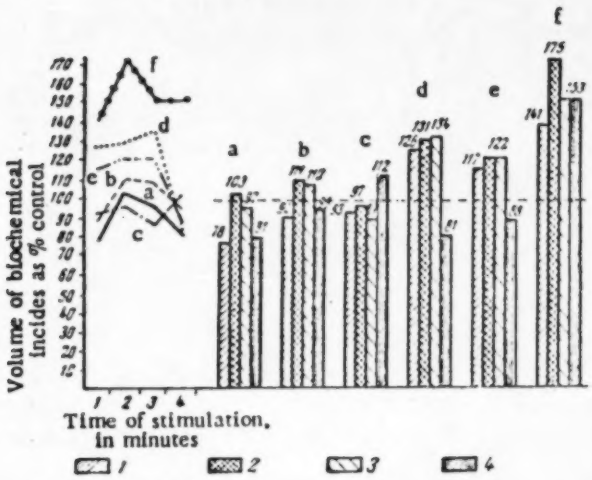

Fig. 1. Changes in biochemical indices of the myocardium with a positive inotropic effect. a) Tissue respiration; b) inorganic phosphorus content; c) phosphocreatine content; d) ATP content; e) phosphocreatine + ATP; f) glycogen content; 1) one-minute stimulation; 2) two-minute stimulation; 3) three-minute stimulation; 4) four-minute stimulation. Broken horizontal line – control (100%).

In the control experiments, biochemical analysis of the heart muscle was carried out without preliminary stimulation of the nerves. At first, an attempt was made to take the control sample and the sample following stimulation from the same heart. However, this had to be abandoned since trauma sustained by the heart in the process of obtaining the control sample resulted in severe changes in biochemical indices, and it was difficult to distinguish such changes from those occurring under the influence of dynamic nerves.

As can be seen from Fig. 1, stimulation of the reinforcing nerve led to changes in all the indices being considered, their nature depending on the duration of nerve stimulation. Thus, tissue respiration, depressed during the first minute of stimulation, reached and maintained the control level during the second and third minutes of stimulation, and again decreased during the fourth minute. Dynamics of the changes in inorganic phosphorus

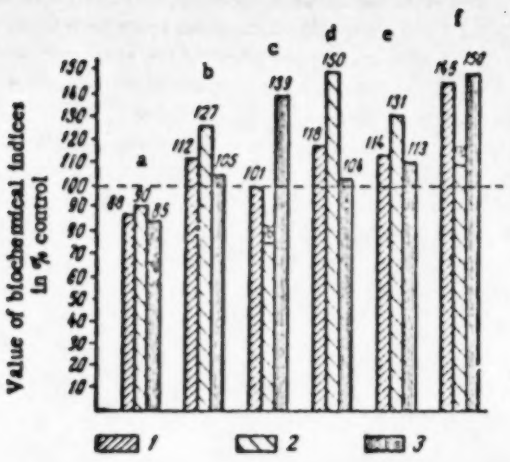

Fig. 2. Changes in biochemical indices of the myocardium with a negative inotropic effect. Legend as that in Fig. 1.

were analogous to those of respiration. Phosphocreatine content remained almost unchanged in the first 3 minutes, and only exceeded the control level to some extent during the fourth minute of stimulation of the reinforcing nerve.

The amount of ATP in the first 3 minutes was above the control level, and was significantly lowered only during the fourth minute. The myocardial glycogen content was increased during stimulation of the reinforcing nerve, reaching a maximum during the second minute.

Unlike the other indices, glycogen content did not drop below the control level throughout the period of stimulation (from 1 to 4 minutes).

The above-mentioned biochemical indices were also examined upon stimulation of the parasympathetic dynamic nerve, which produced weakening of cardiac contractions without slowing of the rhythm. Results of these experiments are presented in Fig. 2.

Figure 2 shows that tissue respiration was below the control level at all values of duration of stimulation. The amount of inorganic phosphorus exceeded the control level appreciably during the third minute. Phosphocreatine, unchanged during the first minute, was lowered during the third minute, and was considerably in excess of control upon four-minute stimulation. The amount of ATP, which increased upon 1-3 minute stimulation of the nerve, returned to control level during the fourth minute. Glycogen content remained above control level throughout.

All the indices studied were thus altered both in the presence of a positive and a negative cardiac inotropic effect.

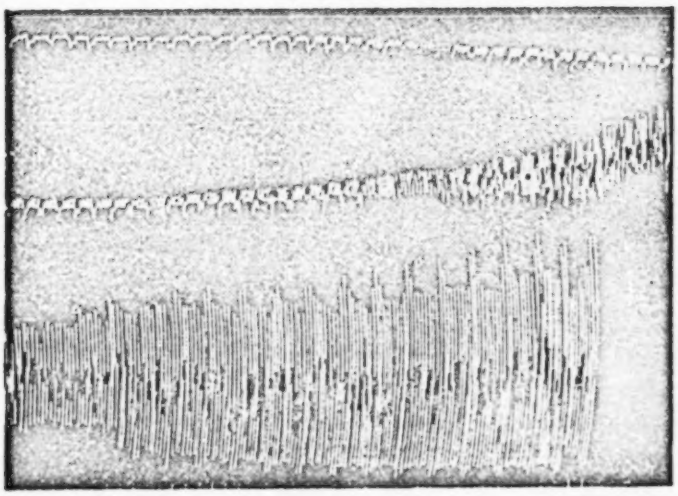

Fig. 3. The effect of reinforcing-nerve stimulation on cardiac activity. Records from above down: cardiac contractions – three tracings, time marker (1 sec): 2 lower tracings are continuation of the top trace.

When the reinforcing nerve is stimulated, the rearrangement of biochemical processes may be directed toward increasing the energy potential of the myocardium. The rapid transition of the heart muscle to increased work leads to respiratory depression, but the total content of ATP and phosphocreatine shows some increase rather than decrease. It may be assumed that more economic use of oxygen and enhancement of the anaerobic phase are involved. The amount of glycogen was altered in the process of stimulation, but remained above the control level without being affected by the duration of stimulation. Increased work following a positive inotropic effect on the heart and increased metabolism appear to result in more intensive breakdown and restoration of glycogen (V. A. Engelhardt's principle); such glycogen supercompensation has been observed in muscular work. All this suggests that the effect of the reinforcing nerve on myocardial metabolism is associated with considerable participation of glycogen. On stimulation lasting 2 and 3 minutes, respiration maintains the control level while the total high-energy bond (~ ph) values and glycogen remain above the control figure. Continuing increased cardiac work under the influence of the reinforcing nerve (on 4-minute stimulation), unaccompanied by increased partial pressure of oxygen, could conceivably lead to fatigue, since respiration decreased, as did the total ~ ph of the ATP.

The character of biochemical changes in the myocardium described was observed in all the experiments involving the reinforcing nerve.

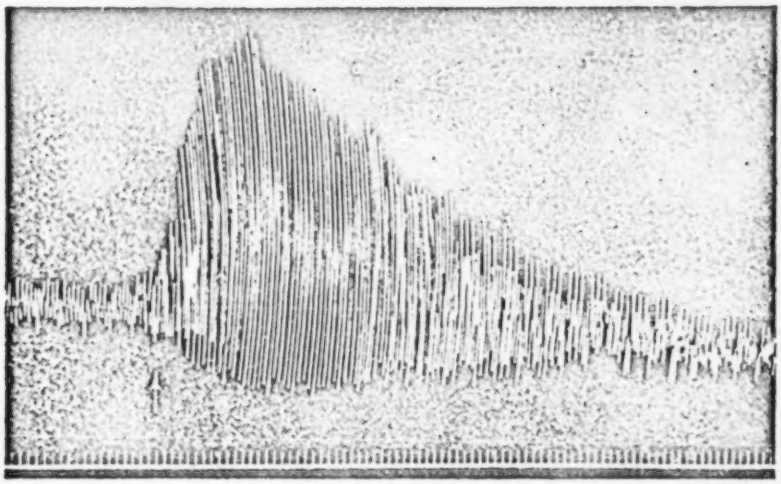

Fig. 4. The effect of reinforcing-nerve stimulation on cardiac activity. Records from above down: cardiac contractions; time marker (1 second).

The degree of changes in individual indices, however, fluctuated in different experiments with a correspondence, in some cases, between the extent to which physiologic effects were pronounced and the intensity of changes in the biochemical indices. In support of this, cardiograms of 2 experiments (Fig. 3 and 4) and corresponding biochemical data are presented.

Figure 3 shows that stimulation of the nerve led to fairly considerable increase in cardiac activity, which was associated with an adequate ~ ph content (117.7%) and glycogen content (296%).

In another experiment, the reinforcing nerve caused transient increase in the amplitude of cardiac contractions, with gradual return to the initial level on continuing stimulation (Fig. 4). Exceedingly low ~ ph (38%) and glycogen (88%) content was found to be associated with this.

The negative inotropic effect of dynamic parasympathetic cardiac nerves is, evidently, accompanied by changes in myocardial trophics in the direction of weakening breakdown processes and enhancing the synthesizing processes. Thus, despite depressed tissue respiration, the total content of ~ ph and glycogen remains above the control level. Thus both sympathetic and parasympathetic cardiac nerves lead to appreciable qualitative rearrangement of myocardial biochemical processes.

SUMMARY

It was demonstrated in experiments on dogs, that the stimulation of dynamic heart nerves of sympathetic and parasympathetic origin (which evoke positive and negative inotropic effects) was associated with the change of contents of phosphorus fractions (inorganic phosphorus, adenosinetriphosphate and phosphocreatine) and, likewise, in the change of intensity of tissue respiration. These changes depended on the duration of the stimulation. The content of glycogen in all cases never went below the control level. The mechanism of these phenomena are discussed in their connection with the physiological effects.

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* In Russian.

** Original Russian pagination. See C. B. translation.

THE ROLE OF ADRENALIN IN THE CHANGE OF BLOOD-SUGAR CONTENT PRODUCED BY ADMINISTRATION OF PHENAMINE

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Phenamine, a preparation synthesized in the USSR, is widely used in medical practice as a nervous system stimulant. It is analogous to benzedrine in its physiologic action.

Investigations [2, 3, 4, 9] have shown that phenamine given in optimal doses (0.01-0.03 g) increases excitability and, apparently, favors concentration of the inhibitory process in the central nervous system.

There are indications [1] that phenamine exerts a tonic effect both on the sympathetic and parasympathetic nervous systems.

Relatively few communications are devoted to the influence of phenamine on blood-sugar content. Those which do deal with this aspect of the preparation give conflicting results. Thus, for example, M. Ya. Sereisky [6] found no appreciable differences when he estimated blood-sugar levels in subjects before administration of phenamine and for some hours (at half-hour intervals) after administration of 0.02 g phenamine. V. V. Vasilyeva [2] noted sometimes increase and sometimes decrease in blood sugar following administration of phenamine. A. M. Timofeeva [8], N.Yu. Belenkov and E. N. Speranskaya [1] found the blood sugar to be increased by administration of the substance. I. S. Kanfor [5] studied the effect of phenamine on diurnal changes in blood-sugar levels in monkeys and also found some increase in the blood-sugar content. N. P. Smirnov [7] investigated the effect of phenamine on blood-sugar levels in dogs with alloxan-induced diabetes, and observed, in the majority of cases, some increase in blood-sugar content 1-2 hours after administration of the preparation.

In view of these conflicting findings, it was decided to undertake another investigation of the influence of phenamine on blood-sugar content. Investigations were carried out on healthy human subjects and on dogs.

METHODS

Fasting blood sugar (8-10 samples every 5 minutes) was estimated in the morning. In the case of human subjects, the blood was taken from the finger; in the case of dogs – from the femoral artery. The sugar was determined by the Hagedorn-Jensen method. It was found that the blood-sugar level was subject to considerable, but not strictly rhythmic, fluctuations. However, the arithmetic mean values for blood-sugar content calculated separately for the first 4-5 samples and for the subsequent 4-5 samples taken in one experiment proved to be very stable (the difference between the mean values did not, as a rule, exceed ± 5 mg%).

Experiments with administration of phenamine were carried out as follows. In the morning, fasting specimens (5-7) of blood were taken every 5 minutes for 30-35 minutes. After this, phenamine was given by mouth. Human subjects received 0.01 or 0.02 g; dogs weighing 18-22 kg received 0.01 g of the preparation. 30-45 minutes after administration of phenamine, blood samples were again taken at short intervals of time.

TABLE 1

The Effect of Phenamine on Blood-Sugar Levels in Healthy Human Subjects and Dogs

Dose of phenamine in g	No. of samples		Arithmetic mean values (mg %)			Maximal amplitudes of fluctuations (mg %)		
	Before administration phenamine	After administration phenamine	Before administration phenamine	After administration phenamine	Difference	Before administration phenamine	After administration phenamine	Difference
Observations on human subjects								
0.01	6	10	90	88	−2	10	9	−1
0.02	6	10	89	90	+1	9	20	+11
0.02	6	14	91	94	+3	6	11	+5
0.02	6	14	90	87	−3	10	20	+10
0.01	6	13	88	80	−8	11	24	+13
0.02	6	13	90	83	−7	5	12	+7
0.02	6	12	96	82	−14	8	16	+8
0.01	6	12	75	71	−4	5	7	+2
0.02	6	13	71	72	+1	9	9	0
0.01	6	15	79	83	+7	13	26	+13
Observations on dogs								
0.01	6	8	80	69	−11	14	6	−8
0.01	5	10	79	78	−1	11	27	+16
0.01	5	6	78	86	+8	12	27	+15
0.01	5	5	69	76	+7	12	30	+18
0.01	5	9	80	79	−1	14	27	+13
0.01	6	10	88	83	−5	13	13	0
0.01	6	6	80	64	−16	12	23	+11
0.01	5	9	85	76	−9	31	39	+8
0.01	6	10	98	113	+15	16	30	+14
0.01	7	7	88	79	−9	21	30	+9
0.01	5	7	90	106	+16	15	42	+27
0.01	6	10	71	79	+8	14	23	+9
0.01	5	7	69	69	0	19	11	−8

In each experiment, the results of blood analysis on samples collected before and after administration of phenamine formed the basis on which separate calculations were made of the arithmetic mean and the maximal differences which were designated conventionally as "maximal amplitude of fluctuation" (Table 1).

RESULTS

Analysis of data obtained revealed the following. The average blood-sugar content was not subject to appreciable changes under the action of phenamine. For example, of 10 investigations carried out on human subjects, any noticeable changes in the average blood-sugar content (exceeding 5 mg%) were obtained in only 4 cases; in three of these the blood sugar content decreased somewhat (by 7-14 mg%); and in one case it increased by 7 mg% following administration of phenamine.

Analogous results were also obtained in experiments on dogs. Of 13 experiments, four showed no noticeable changes in the average blood-sugar content. In 5 experiments, there was some rise in blood-sugar level (by 7-15 mg%), and in 4 experiments a fall of 7-16 mg% following administration of the preparation was observed.

In all cases the changes were within the limits of normal blood-sugar content.

The data presented also show that administration of phenamine, without appreciably altering the blood sugar content (mean values), leads to some increase in the fluctuations of its content compared with fluctuations

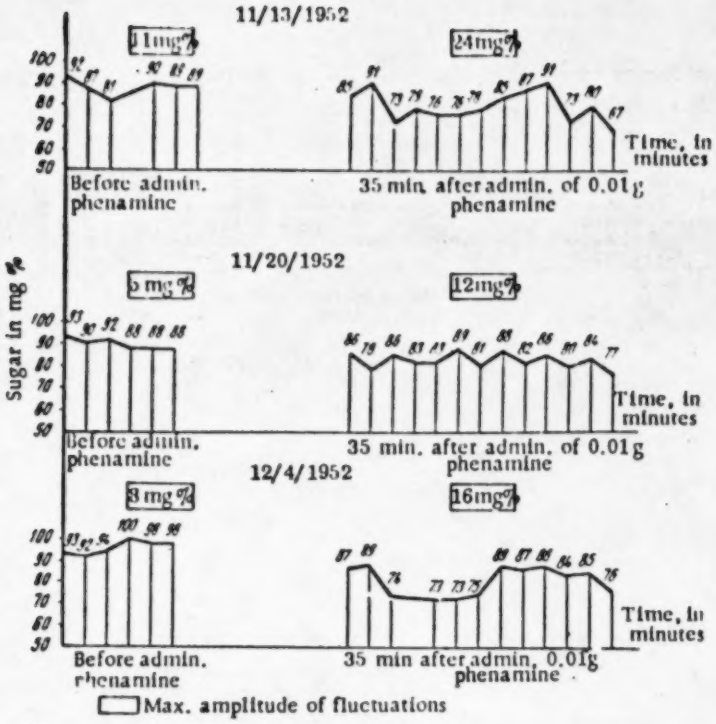

Fig. 1. Effect of phenamine on fluctuations in blood sugar over short intervals of time in subject Y.

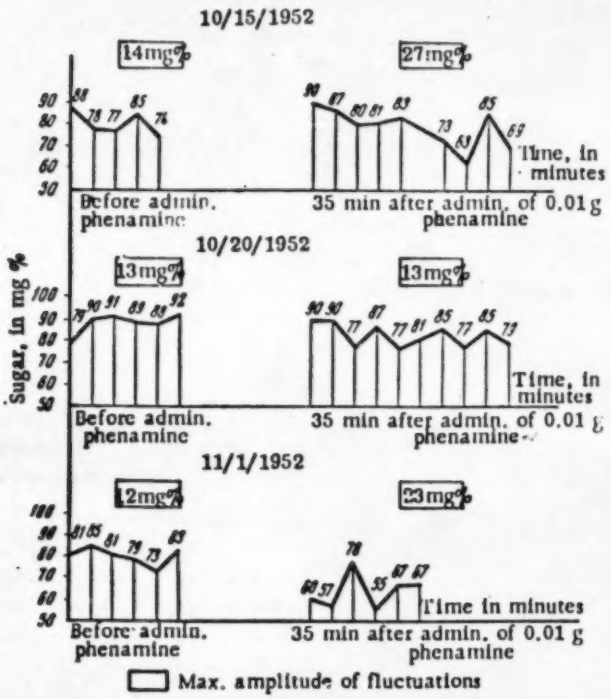

Fig. 2. The effect of phenamine on fluctuations of blood-sugar content over short intervals of time in a dog.

in the same individual on the same day before administration of phenamine. Of 10 human experiments, marked increase of "maximal amplitude of fluctuations" – by 7-10 mg% – was noted in six.

Experiments on dogs gave still clearer results.

An increase in the "maximal amplitude of fluctuations" was obtained in 10 of 13 cases and reached 13, 14, 16, 18 and even 27 mg%. In one experiment, noticeable changes were absent, while in two the extent of fluctuations was somewhat decreased (by 7 and 8 mg%).

Thus, in 16 of 23 experiments, the extent of fluctuations of blood-sugar content was increased after administration of phenamine; some decrease was noted in only 2 cases; and no appreciable changes were observed in 5 cases.

TABLE 2

The Effect of 0.01 g Phenamine on Blood-Sugar Levels in Dogs Following Denervation of the Liver and Adrenals

Experimental conditions	No. of experiment	No. of samples		Arithmetic mean values in mg%			Maximal amplitudes of fluctuations in mg%		
		before admin. of phenamine	after admin. of phenamine	before admin. of phenamine	after admin. of phenamine	Difference	before admin. of phenamine	after admin. of phenamine	Difference
Denervation of liver	1	6	11	93	102	+ 9	24	33	+ 9
	2	8	9	79	93	+14	20	32	+12
Denervation of liver, removal of one and denervation of other adrenals	1	9	10	71	73	+ 2	28	23	− 5
	2	9	10	77	76	− 1	11	13	+ 2
	3	7	9	83	85	+ 2	17	17	0
	4	8	8	83	73	−10	17	22	+ 5
Removal of one and denervation of other adrenals	1	7	10	77	74	− 3	16	19	+ 3
	2	8	10	73	71	− 2	13	14	+ 1
	3	8	10	76	73	− 3	13	18	+ 5
	4	7	6	72	72	0	20	28	+ 8

A study of the graphs draws attention to the marked shortening of time between successive rises in blood-sugar level evoked by phenamine in a number of cases; in other words, the rhythm of fluctuations is markedly accelerated (Figs. 1 and 2).

These changes in blood-sugar level fluctuations evidently result from reflex effect on organs directly involved in the maintenance of blood sugar at a definite level.

In this connection, attention should be directed first of all towards the liver and particularly the adrenals, since it is well known that there is direct connection between excitation of the CNS and reflex adrenalinemia. With this in mind, we carried out two preliminary experiments on animals with partially denervated livers.

The operation consisted of removal of all tissues, including the nerves, constituting the hepato-gastric-duodenal ligament. Only the blood vessels and bile duct were left intact. The animals were allowed to recover from the surgical procedure, after which an experiment with administration of phenamine was performed on each animal. This led to increased fluctuation of the blood-sugar content in both animals ("maximal amplitudes of fluctuations" increased by 9 and 12 mg%). There was also some increase in the average blood-sugar content (Table 2).

Interruption of the main nerves leading to the liver thus did not abolish the characteristic phenamine effect expressed in increasing range of blood-sugar content fluctuations.

To elucidate the situation further, we subjected two dogs with denervated livers to a second operation aimed at preventing the possibility of reflex adrenalinemia. The operation consisted of removal of the right and denervation of the left adrenals. When the animals had recovered from surgery they were used for experiments with administration of phenamine. Four such experiments were staged on 2 dogs. In all 4 experiments, administration of phenamine failed to produce increased amplitude of fluctuation of blood-sugar levels. The mean blood-sugar content also remained substantially unchanged (Table 2).

Further investigations were carried out on a dog with intact innervation of the liver, but with the possibility of reflex adrenalinemia removed (removal of right and denervation of left adrenals). Four experiments with administration of phenamine were performed on this dog. In three of these, no increase in the amplitude of fluctuations of blood-sugar content was noted. The difference between the "maximal amplitudes of fluctuation" did not exceed 5 mg%, i. e., the possible experimental error. Only in one experiment did the "maximal amplitude of fluctuations" increase by 8 mg% (Table 2). The mean blood-sugar content in all 4 experiments remained virtually unchanged.

It can thus be considered that exclusion of reflex adrenalinemia prevents increased fluctuations in blood-sugar content which occur in healthy animals on administration of phenamine and that adrenalin participates in changes in blood-sugar levels evoked by administration of phenamine. Under these conditions, however, increased secretion of adrenalin by the suprarenals does not lead to stable hyperglycemia but is reflected only in the degree of blood-sugar level fluctuations.

SUMMARY

The effect of phenamine on the changes of blood-sugar level was studied in healthy people as well as in dogs. 5-7 blood tests were performed at 5-minute intervals. Then the dose of 0.01-0.02 g of phenamine was introduced. In 30-45 minutes blood tests were taken again at 5-minute intervals. Blood-sugar level was determined by Hagedorn's and Jensen's method in 6-10 specimens of blood, thus obtained. Analysis of data showed that phenamine introduction causes increased variations of blood-sugar level and acceleration of the rhythm of these variations. Partial denervation of liver in dogs (removal of all nerves passing through the gastro-hepatic-duodenal ligament) is not reflected in increased variations of glycemic level, which appears in healthy animals after phenamine introduction. If the possibility of reflex adrenalinemia is excluded (by removal of right and denervation of the left suprarenal gland), there is no increase in the variations of contents of sugar in the blood after introduction of phenamine in animals.

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PHARMACOLOGY

THE EFFECT OF EPHEDRINE ON CAROTID CHEMORECEPTORS

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Studies of the effect of various chemical agents on the isolated carotid sinus helped to elucidate the important part played by the latter in regulating the organism's activity (C. Heymans, S. V. Anichkov, G. Liljestrand and others).

Among other substances, sympathomimetic agents have also been used.

Thus, S. V. Anichkov, V. V. Zakusov, A. I. Kuznetsov and N. G. Polyakov (1936) noted the excitatory action of adrenalin on respiration mediated by carotid sinus chemoreceptors.

A. I. Kuznetsov (1938) noted that on perfusion of the isolated carotid sinus with a 1:100,000 solution of adrenalin, there was stimulation of respiration and lowering of blood pressure. Similar action has been exhibited by sympatol (1:1000).

C. Heymans and D. Cordier (1940) point out respiratory changes which occur under the influence of adrenalin and which are mediated by reflexes from the carotid sinus zone.

C. Heymans (1953) studied the effect of adrenalin and noradrenalin on blood pressure from the point of view of the carotid sinus zone. S. V. Anichkov (1951) studied the effect of adrenalin on the carotid sinus chemoreceptors and referred to it as a feebly acting agent.

A. I. Kuznetsov, studying the effect of ephedrine on the isolated carotid sinus, noted stimulation of respiration during perfusion with ephedrine in 1:100,000 dilution. He considers that ephedrine, like other sympathetic poisons, exerts a weak action on carotid chemoreceptors.

C. Heymans (1953) observed changes in blood pressure under the action of ephedrine on the carotid sinus.

Although some data are available concerning the action of ephedrine on the carotid body, its effect on the sensitivity of carotid body cells to various chemical substances has not been studied at all. Changes in sensitivity of carotid chemoreceptors are of no mean importance to reflex regulation of vegetative functions of the organism.

It is well known that ephedrine increases the sensitivity of adrenalin-reactive systems to adrenalin. Our investigations (A. I. Mitrofanov, 1956) have shown that ephedrine abolishes the blocking effect of ganglion-blocking substances on n-choline -reactive systems of ganglia which have common pharmacologic features with those of the carotid bodies.

METHODS

An investigation (on 31 cats) was made of the effect of ephedrine on the sensitivity of carotid body chemoreceptors to acetylcholine and to sodium cyanide.

Isolated carotid sinus (Moiseev-Heymans-Anichkov technique) was used. Respiration served as test reaction.

RESULTS

Perfusion with ephedrine solutions of various concentrations (1:16,666; 1:50,000 and 1:500,000) did not produce any definite changes in respiration. These data differed from those of A. I. Kuznetsov (1938) who observed stimulation of respiration under the influence of ephedrine in 1:100,000 dilution.

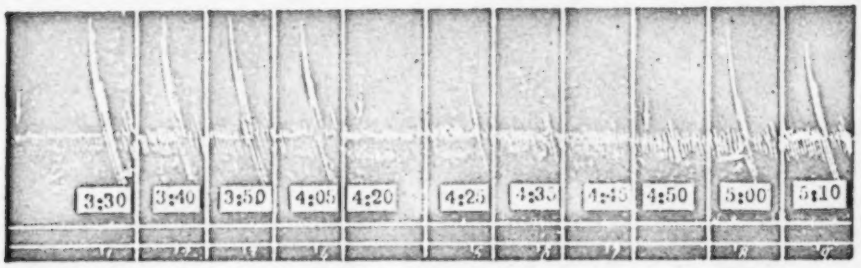

Fig. 1. Effect of ephedrine on carotid chemoreceptors. Perfusion of carotid sinus by the Moiseev-Heymans-Anichkov technique. Records from above down: respiration, time marker ↓ introduction of Ringer-Locke solution; introduction of ephedrine solution 1:16,666; 1, 2, 3, 4, 5, 6, 7, 8, 9) introduction of acetylcholine solution 0.4 ml 1:25,000. Experiment: March 2, 1955. Decerebrate cat, 3.6 kg.

At the same time our experiments showed that the sensitivity of the carotid body to acetylcholine (0.4 ml 1:25,000) was lowered under the influence of ephedrine (in various concentrations); the degree of lowering was related to dilution of ephedrine. Reactivity of the carotid body was restored on washing with Ringer-Locke solution.

Figure 1 demonstrates that the respiratory reaction to the first and to repeated administration of acetylcholine during perfusion with Ringer-Locke solution is practically identical. The reaction to acetylcholine is diminished when perfusion with ephedrine solution (1:16,666) is begun, and by the 15th minute of such perfusion it virtually disappears. During washing with Ringer-Locke solution, the respiratory reaction to acetylcholine is restored, but does not reach the initial value even by the 20th minute.

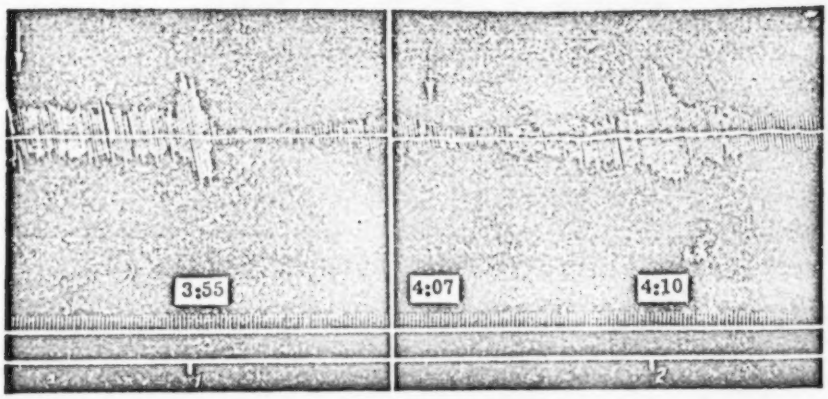

Fig. 2. Effect of ephedrine on carotid chemoreceptors. Perfusion of carotid sinus by the Moiseev-Heymans-Anichkov technique. Records: similar to Fig. 1. ↓ introduction of Ringer-Locke solution; ↓ introduction of ephedrine solution 1:50,000; 1, 2) introduction of sodium cyanide solution 0.2 ml 1:1000. Experiment March 18, 1955. Decerebrate cat, 2.8 kg.

Changes in sensitivity of the carotid body to acetylcholine under the influence of ephedrine could develop as a consequence of vasoconstriction in the carotid body with resultant hindrance of acetylcholine access to its chemoreceptors. To check this, we staged experiments in which sodium cyanide – the anoxic poison being studied – was introduced into the inlet tube during perfusion with ephedrine. If diminution of reaction to acetylcholine were due to vasoconstriction, there should also be diminution of reaction to cyanide, all other conditions being similar. Since 1 : 50,000 concentration of ephedrine was found experimentally to give a clear, but not extremely pronounced effect, it was this concentration which was used in subsequent experiments.

Sodium cyanide in amounts of 0.2 ml (1 : 1000) increased respiratory reaction when given during perfusion with ephedrine (1 : 50,000).

Figure 2 shows that ephedrine (1 : 50,000) not only does not weaken the carotid body reaction to cyanide, but, on the contrary, evokes certain enhancement of this reaction (judged by stimulation of respiration) as compared to the original.

The experiments thus demonstrated that vasoconstriction in the carotid body during perfusion with ephedrine was hardly likely to play a part in the diminished reaction to acetylcholine.

It was essential to check that sensitivity of the carotid body to acetylcholine was diminished, and its sensitivity to sodium cyanide increased, during perfusion with ephedrine under identical conditions (on the same animal). Into the inlet tube was introduced 0.4 ml acetylcholine solution (1 : 25,000) and 0.2 ml sodium cyanide solution (1 : 10,000). Lower concentration of sodium cyanide than in the previous experiments was taken intentionally, in order to evoke a smaller change in respiration than that produced by acetylcholine. Acetylcholine was introduced first, followed by cyanide after 5-10 minutes. This was prompted by the fact that preliminary introduction of acetylcholine did not alter the action of sodium cyanide. Changes in respiration obtained under these experimental conditions are presented in Fig. 3, which shows that normally sodium cyanide (0.2 ml 1 : 10,000) alters respiration much less markedly than acetylcholine (0.4 ml 1 : 25,000). During perfusion with ephedrine,

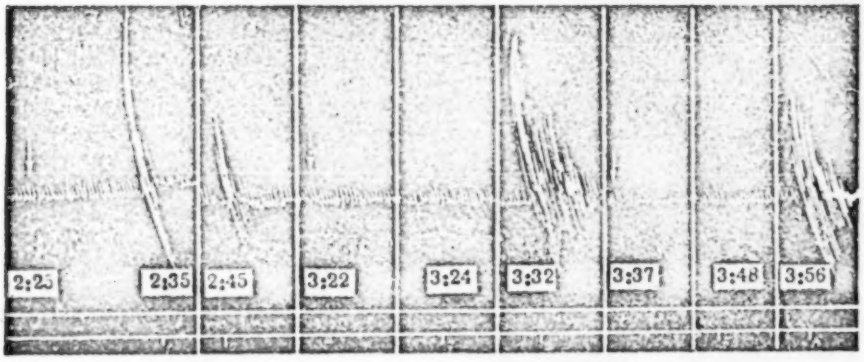

Fig. 3. Effect of ephedrine on carotid chemoreceptors. Perfusion of carotid sinus by the Moiseev-Heymans-Anichkov technique. Records: similar to Fig. 1. ↓ introduction of Ringer-Locke solution; ↓ introduction of ephedrine solution 1 : 50,000; 1, 3, 5) introduction of sodium cyanide solution 0.2 ml 1 : 10,000. Experiment March 28, 1955. Decerebrate cat, 3 kg.

the carotid body sensitivity to acetylcholine drops sharply, while sensitivity to sodium cyanide increases significantly (judging by stimulation of respiration). When ephedrine is washed away with Ringer-Locke solution its sensitivity to acetylcholine begins to increase slightly and its sensitivity to cyanide begins to decrease.

These experiments show that ephedrine causes some biochemical shifts to occur in the carotid body, which lead to changes in its reaction to acetylcholine and cyanide.

It is known that carotid body chemoreceptors in some respects approach the n-choline-reactive systems of ganglia, as witnessed by the selective action of ganglion poisons both on the carotid body and the sympathetic ganglion (S. V. Anichkov and collaborators, 1936; S. V. Anichkov, 1945, 1947).

Taking into account the results obtained, it was essential to discover whether the antagonism between ephedrine and anticholinergic substances would not become apparent in their action on the choline-receptors of the carotid body. This investigation was of theoretical as well as practical interest, considering the physiological role of the carotid body.

Paramion* was used as an anti-n-choline agent; it is a curare-like substance. It is known that curare blocks carotid chemoreceptors; the effect of paramion on the carotid sinus had not been studied up to the present.

No significant changes in respiration were noted during perfusion of the isolated carotid sinus with paramion in concentrations of 1 : 10,000 and 1 : 5000; carotid body sensitivity to acetylcholine was lowered, indicating blocking of n-choline-reactive systems in the carotid body. Washing with Ringer-Locke solution led to restoration of this sensitivity, which was fairly rapid (by the 3rd minute).

Paramion did not alter carotid body sensitivity to sodium cyanide (0.2 ml 1 : 1000 in inlet tube).

It is known that curare, too, does not exert any effect on carotid body sensitivity to anoxic poisons, in particular the cyanides (S. V. Anichkov, 1949, 1953).

Paramion thus exerts the same effect on the carotid body as curare.

Having established the changes in sensitivity of the carotid body under the influence of ephedrine alone, and paramion alone, we undertook the study of their combined effect.

Perfusion with a solution containing a mixture of ephedrine (1 : 50,000) and paramion (1 : 5000) was associated with marked weakening of the respiratory reaction, indicating blocking of the carotid body n-choline-reactive systems.

Restoration of carotid body sensitivity to acetylcholine after washing with Ringer-Locke solution was delayed as compared to such restoration after perfusion with ephedrine or paramion, separately, and subsequent washing with Ringer-Locke solution.

No antagonistic action of ephedrine and paramion on the carotid body chemoreceptors was therefore found in our experiments. These results did not agree with data from experiments on n-choline-reactive systems of autonomic ganglia where antagonism between ephedrine and anticholinergic agents, with a competitive type of action, was found to exist (A. I. Mitrofanov, 1956).

At the same time, the present experiments showed that ephedrine enhanced carotid body sensitivity to cyanides. Evidently, ephedrine increases carotid body sensitivity to oxygen deficiency, i. e., enhances its participation in regulation of oxygen content in the blood.

SUMMARY

The carotid sinus was isolated by the method of Moiseev-Heymans-Anichkov, respiration serving as a test. Perfusion of 1 : 50,000 solution of ephedrine caused respiration to be lowered by acetylcholine and enhanced by the sodium cyanide. Thus, it has been shown that ephedrine accelerates sensitivity of the carotid body to the cyanides.

Cyanides are anoxic poisons, their effect being like that of oxygen insufficiency. Hence, ephedrine enhances the sensitivity of the carotid body to oxygen insufficiency accelerating its participation in the control of blood oxygen.

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THE EFFECT OF CERTAIN TYPES OF NOVOCAINE BLOCK ON CONDITIONED FOOD REFLEXES

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(Received March 15, 1957. Presented by Active Member USSR Academy of Medical Sciences, Professor V. N. Chernigovsky)

The work of Professor A. V. Vishnevsky is of fundamental importance in the studies of the mechanism of the therapeutic effect of novocaine block; he regards novocaine as a weak nervous system stimulant under whose influence changes in the neurotrophic function of the nervous system may occur. Even prior to discovery of novocaine it had been found that injection of anesthetic substances could give rise not only to analgesic but also therapeutic effects, as mentioned by S. P. Protopopov [13].

However, the mechanism of action of novocaine block on the organism still requires detailed study and explanation in view of its great complexity.

A number of authors [1, 4, 11 and others] have observed that various types of novocaine block give rise not only to local analgesia but often also to drowsiness and even sleep.

In recent times more and more references are encountered in medical and surgical literature [1, 14, 15 and others] to the question of using intravenous novocaine block as prophylaxis for surgical shock. This is based on the hypothesis that novocaine, acting on vascular receptors, interrupts the flow of pain impulses and stimuli to the central nervous system and so protects the organism from development of shock. In favor of this view are M. A. Bubnov's [2] data which showed that preoperative intravenous injection of novocaine led to enhancement of inhibitory processes in the CNS and to lowering of its functional lability. The influence of novocaine on the reflex function of the nervous system has been studied by many authors. Most of them [5, 6, 7, 8, 9, 12 and others] maintain that novocaine causes depression and even complete abolition of both exteroceptive and interoceptive unconditioned and conditioned reflexes. According to E. I. Shur [16] and E. I. Shur, L. A. Baraz, R. S. Vinnitskaya and V. I. Popov [18], who studied interoceptive reflexes following lumbar novocaine block by Vishnevsky's method, the transient depression was followed by enhancement of reflexes to blood pressure and respiration on stimulation of intestinal chemoreceptors and large intestine and urinary bladder mechanoreceptors. Moreover, E. I. Shur [17] reports that more marked enhancement of reflexes from the interoceptors mentioned above was noted against the background of aseptic inflammation of a region of the small intestine.

It is thus seen that opinions concerning the effect of novocaine on the reflex function of the nervous system are to some extent contradictory.

We undertook the study of the effect of various types of novocaine block on the cerebral cortex using conditioned salivating reflex technique in this investigation.

METHODS

Experiments were performed during 1954-1955 in a conditioned reflex chamber; three dogs (Bars, Silva and Galka) with salivary fistulas of the parotid glands were used. Conditioned salivating reflexes were established

to a bell in the case of Silva and Bars; 29 combinations were required for the former and 101 combinations for the latter; in the case of Galka a conditioned reflex was obtained to metronome clicks at the rate of 100 per minute after 20 combinations. The conditioned stimulus was applied for 20 seconds. Rusk powder (3 g) served as unconditioned stimulus. Following establishment of the conditioned reflexes in Bars and Silva differentiation to a bell of lower tone (bell B) was obtained, in Silva's case after 23 trials and in the case of Bars after 56 trials; differentiation to M_{40} was obtained in Galka after 96 trials. On this background of these conditioned reflexes and differentiations the action of spinal, intraperitoneal and paranephritic block was investigated.

On the day before administration of novocaine the magnitude of the conditioned reflex and differentiations was determined using the stimuli referred to above. The next day local anesthesia was induced and the conditioned reflexes tested anew. 24 hours later a control experiment was carried out to compare the magnitude of the conditioned reflexes with data obtained in the preceding experiment. Quite often changes in conditioned reflexes were checked for 2-4 days after the use of novocaine block.

A total of 172 series experiments were performed on Galka, 245 on Silva and 252 on Bars. This includes 49 experiments with investigation of various types of novocaine block.

RESULTS AND DISCUSSION

For spinal (sacral and lumbo-sacral) anesthesia, warmed 1.5% solution of novocaine was used; it was introduced in amounts of 6-12 ml between coccygeal vertebrae I and II or between the last lumbar and first sacral vertebra with evidence of marked depression and even complete abolition of conditioned reflexes after only 10-15 minutes (Fig. 1).

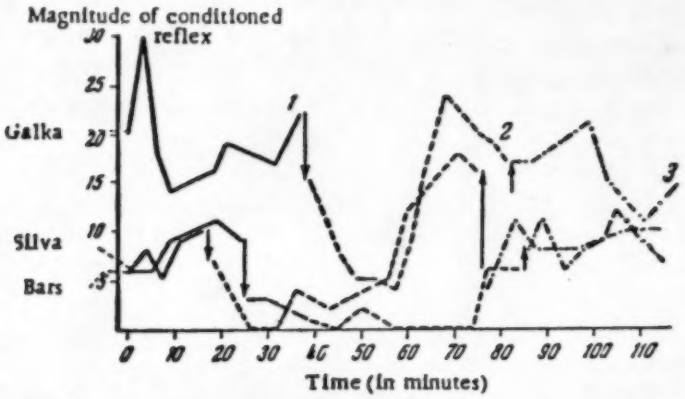

Fig. 1. Changes in magnitude of conditioned reflexes with epidural anesthesia.
1) Day before administration of novocaine; 2) day of experiment with novocaine;
3) 24 hours after administration of novocaine.

The reflexes remained depressed for 30 minutes on the average and only in one experiment they remained depressed for up to one hour following which they were completely restored; sometimes the magnitude of the reflexes was even greater than the initial value towards the end of an experiment. 24 hours after epidural anesthesia the conditioned reflexes had returned to preblock values. Characteristically, when this type of local anesthesia was used the orientation reaction to the conditioned stimulus was diminished or disappeared altogether. The animal succumbed to a state of drowsiness. As the action of novocaine wore off the orientation reaction reappeared and a parallel restoration of the conditioned reflex was then observed.

In using intraperitoneal anesthesia a comparison was made of the action of sovcaine* in dilutions of 1:1000 and 1:500 and of 1% solution of novocaine. The most marked depressive action in this type of anesthesia was produced by the 1:1000 solution of sovcaine which was given in a dose of 60 ml. Depression of the conditioned reflexes supervened not earlier than 40-45 minutes after administration of novocaine, lasting on

* Russian trade name.

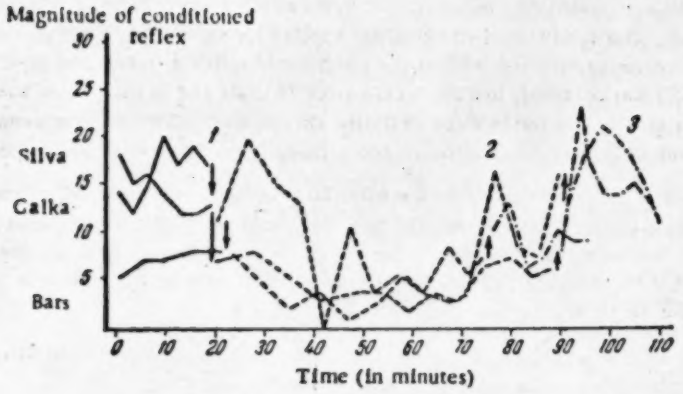

Fig. 2. Changes in the magnitude of conditioned reflexes with intraperitoneal anesthesia. Legend similar to Fig. 1.

the average 30-32 minutes (from 20 to 50 minutes), the value of conditioned reflex falling to zero and then gradually reaching normal levels. Sovcaine in dilution of 1:500 produced depression of reflexes after 5-10 minutes but since its action was accompanied by manifestations of intoxication – vomiting, defecation, extremely copious salivation – its use was rejected.

Depression of reflexes following administration of 1% solution of novocaine given in doses of 30 ml occurred after an average of 29 minutes and lasted from 20 to 49 minutes, an average of 34 minutes (Fig. 2). The extent of depression in this case was, however, rather smaller than in the case of sovcaine.

When intraperitoneal anesthesia was used the reflexes were not always restored to initial values by the end of experiment: in a number of cases the reflexes remained diminished and only reached the initial magnitude during the next experiment.

In the absolute majority of cases the orientation reaction to the conditioned stimulus was preserved; the animal turned its head towards the stimulus, looked alert, licked its lips, stretched towards the food container, the reflexes remaining depressed or even absent.

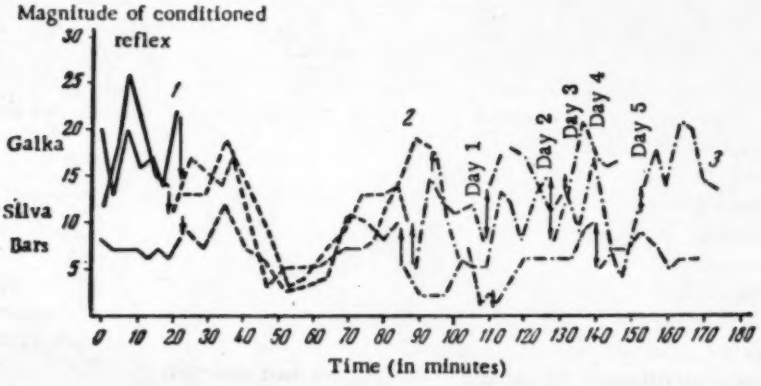

Fig. 3. Changes in the magnitude of conditioned reflexes with paranephritic block. 1) Day before administration of novocaine; 2) day of experiment with administration of novocaine; 3) 24-72 hours after administration of novocaine.

Paranephritic block was achieved by administration of 30 ml 0,5% novocaine next to the transverse process of the second lumbar vertebra on the left side. Silva showed depression of conditioned reflexes after 35-45 minutes, lasting up to 40 minutes and on one occasion even an hour. Galka showed very similar effects: depression occurred after 37-40 minutes and lasted about 40 minutes. Depression of reflexes occurred more quickly in Bars – after 20 minutes – and lasted from 25 to 40 minutes. The magnitude of the conditioned reflex dropped to 2-3 divisions of the record scale and in some cases fell to zero (Fig. 3).

Unlike intraperitoneal anesthesia, paranephritic block was not, in most cases, followed by restoration of conditioned reflexes to their initial values by the end of experiment.

Worthy of attention is the fact that after paranephritic block the conditioned reflexes remained depressed on the second and even third day following the block and only reached initial values on the fourth day. This had been observed earlier by T. S. Miryushchenko [10].

While investigating the various types of novocaine block we wondered whether the animal might not develop a conditioned reflex to the experimental conditions such as needle prick during injection, etc. and the observed depression of conditioned reflexes thus being not a consequence of the effect of novocaine but a result of the action of completely extraneous factors in no way connected with the local anesthesia. It was therefore decided to substitute warmed physiologic solution for novocaine. Such experiments were performed after spinal and intraperitoneal anesthesia. Physiologic solution was administered in the same amounts as novocaine 1-2 days after novocaine block. No changes of the magnitude of conditioned reflexes were noted under the influence of physiologic solution (Fig. 4).

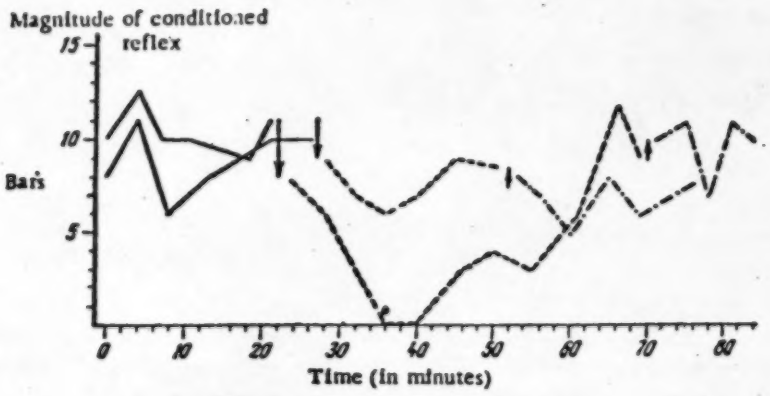

Fig. 4. Control experiment with introduction of physiologic solution. Legend similar to Fig. 1.

The experiments described thus demonstrated that novocaine block exerted an influence on the magnitude of conditioned reflexes.

As regards the mechanism underlying novocaine block of various types it may be supposed that exclusion of large numbers of interoceptors and exteroceptors by novocaine diminishes the inflow of impulses to the central nervous system and thus alters the functional state of the cerebral cortex.

Under these conditions inhibition can readily develop with consequent depression and even disappearance of conditioned reflexes as has been observed in the present experiments.

This hypothesis is confirmed by the fact that with epidural anesthesia which blocks the greatest number of receptors the effect observed was the most pronounced.

Changes with intraperitoneal and paranephritic block were less marked and the effect was seen later than in epidural anesthesia, viz. after 30-40 minutes. This is evidently explained by the heterogeneous character of the receptive fields acted upon by novocaine depending on the mode of its administration.

The data obtained confirm the general action of novocaine which alters the functional state of the central nervous system including that of the cerebral cortex.

SUMMARY

The influence of various types of novocaine block on conditioned food reflexes were studied. Paranephritic, spinal, and intraperitoneal introduction of novocaine and sovcaine was used. Depression of conditioned reflexes was revealed, but orientating reaction was preserved. The degree of this depression and its duration were different in various methods of novocaine introduction. Depression was grestest in epidural introduction. Probably, this effect is connected with extinguishing of afferent impulses by novocaine.

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THE EFFECT OF PARENTERAL ADMINISTRATION OF PAS ON UNCONDITIONED INTEROCEPTIVE REFLEXES

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PAS occupies an extremely important place among chemotherapeutic agents used successfully in the treatment of tuberculosis. The mechanism of its action, however, has as yet been inadequately investigated.

In the course of studies on the pharmacologic properties of PAS we had established [2] that PAS evoked, in the first phase of its action, stimulation of chemoreceptors on direct contact with them while in the second phase of its action it produced reversible functional "extinction" of chemoreceptors.

In the present communications, data are reported concerning the effect of PAS on interoceptive reflexes under conditions of resorptive action of the substance given intramuscularly.

METHODS

Experiments were carried out on cats under urethane anesthesia and also on decerebrate animals (a total of 64 experiments). Blood pressure and respiratory reflexes were studied.

In the first series of experiments, a study was made of the effect of PAS on reflexes from chemoreceptors of a segment of the small intestine humorally isolated and connected with the organism by nerves only.

At the start of an experiment, chemoreceptor reflex reactions to acetylcholine and carbonic acid in the perfused segment of intestine were established. PAS was then given intramuscularly (0.25-1 g per 1 kg body weight), following which reflexes arising on addition of the above-mentioned substances to the perfusate were tested every 15 minutes for an average of $1\frac{1}{2}$ hours.

RESULTS

The experiments showed that parenteral administration of PAS led, in the majority of cases, to diminution of blood pressure reflexes and partly of respiratory ones evoked by stimulation of the chemoreceptors in the perfused segment of small intestine. In a number of experiments the reflexes disappeared completely (Fig. 1). Enhancement of reflexes elicited by stimulation of chemoreceptors was observed only in those experiments in which acetylcholine in high concentration ($1 \cdot 10^{-3}$) was used as stimulus. Of 8 experiments in which acetylcholine in this concentration was used, complete suppression of blood pressure reflex was observed in one; in two, the initial reflex was diminished by 30-25%; and in 5 experiments administration of PAS was followed by increase in reflexes.

Of 13 experiments in which reaction to acetylcholine in concentration of $1 \cdot 10^{-4}$ was studied, in two the reflex disappeared completely; in 5 experiments considerable depression of the reflex was observed (by 92-41.6%). Less marked inhibition of the reflex was seen in 4 experiments (by 36-25.6%), and only in 2 experiments was the reflex enhanced following administration of PAS.

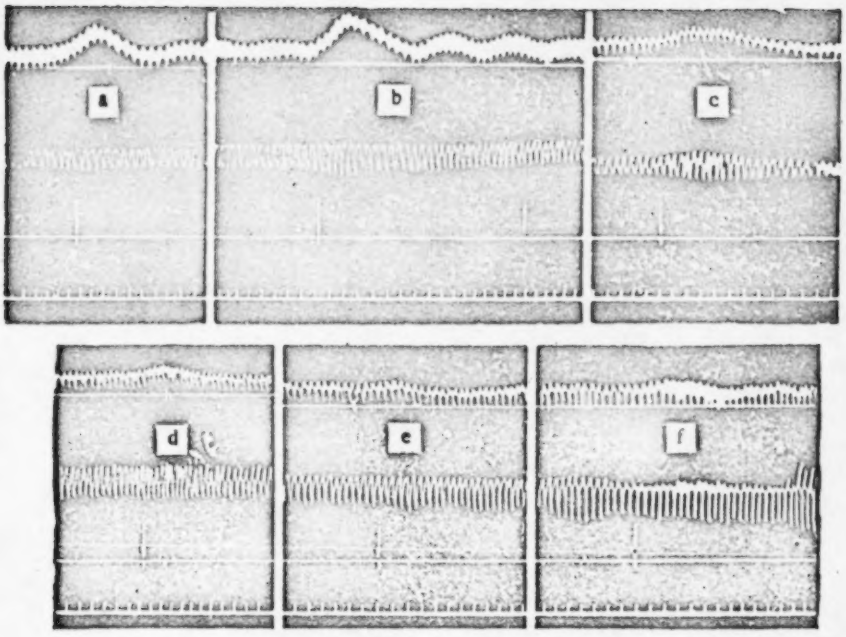

Fig. 1. Effect of intramuscular administration of PAS on reflexes from chemoreceptors in perfused segment of small intestine. a, b) Initial reflexes; c, d, e, f) reflexes after administration of PAS (0.5 g per 1 kg weight). Records from above down: blood pressure, respiration, stimulus marker, time marker (5 seconds). Arrow indicates time of PAS injection.

Of 4 experiments in which carbonic acid was used as chemoreceptor stimulant, in one the blood pressure reflex disappeared altogether; in two it was decreased by more than 50%; and in one – by 42.8%.

Since experimental conditions excluded the possibility of direct action by PAS on the chemoreceptors in the segment of intestine it could be assumed that the preparation, given intramuscularly, affected the central components of the interoceptive reflex arc. An attempt was made to localize more precisely the site of PAS action in the interoceptive reflex arc in the second series of experiments in which urinary bladder mechanoreceptors were subjected to excitation. This was achieved by distention of the bladder with air introduced into it under a pressure of 80-100 mm Hg. PAS was given intramuscularly in the dose of 0.5 g per 1 kg body weight.

The results of these experiments were as follows. PAS in the dose that consistently inhibited reflexes from chemoreceptors in the isolated loop of small intestine did not, as a rule, inhibit reflexes from bladder mechanoreceptors. In 5 out of 12 experiments, enhancement of blood pressure reflex was observed as soon as 15 minutes after injection of PAS. In 4 other experiments, enhancement of the reflex was preceded by its slight decrease over a period of 30 minutes to 1 hour; the reflex then gradually increased, finally exceeding the initial value. In 2 experiments the reflex remained unchanged, and in only one experiment was it diminished.

These experiments indicate that the vasomotor and respiratory centers, and the afferent pathways leading from them to blood vessels and heart, and also to respiratory musculature are not subject to the inhibitory effect of PAS, since otherwise the reflex from urinary bladder mechanoreceptors could not have been realized.

In order to confirm this conclusion, we staged a third series of experiments totalling 12, in which the effect of PAS on unconditioned reflexes arising from chemo- and mechanoreceptors was traced within the same experiment. Reflexes from small intestine chemoreceptors (to acetylcholine in concentration of $1 \cdot 10^{-4}$) and carbonic acid), from urinary bladder mechanoreceptors, and from receptors of the carotid sinus reflexogenic zone were studied at different times before and after injection of PAS. Observations in this series confirmed the findings

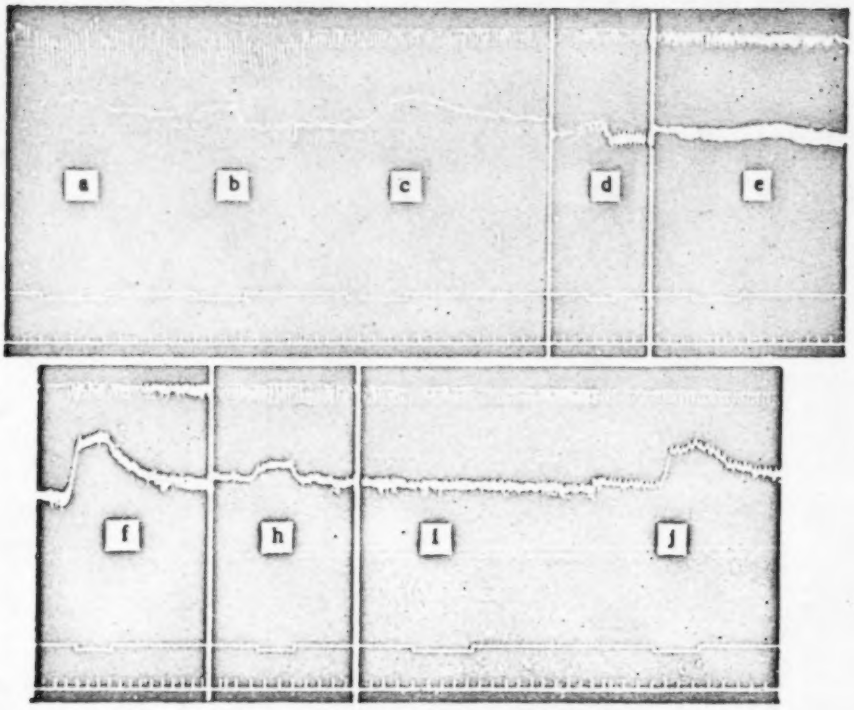

Fig. 2. Effect of PAS on reflexes from small intestine chemoreceptors, carotid sinus receptors and urinary bladder mechanoreceptors. a, b, c) Initial reflexes after injection of PAS (1 g per 1 kg weight); e) diminution; i) complete inhibition of reflexes from small intestine chemoreceptors; d, h) diminution of reflexes from carotid sinus receptors; f, j) enhancement of reflexes from urinary bladder mechanoreceptors. Records same as in Fig. 1.

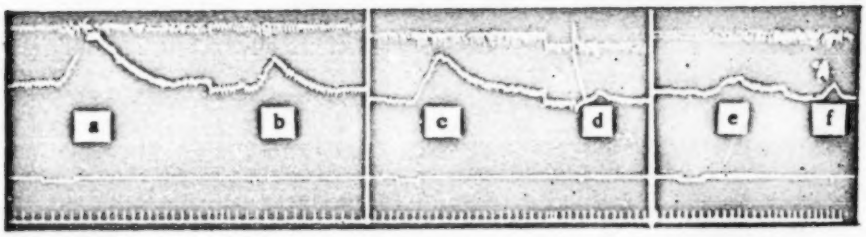

Fig. 3. Effect of PAS on reflexes from small intestine chemoreceptors in decerebrate cat. a, b) Initial reflexes; c, d, e) reflexes after injection of PAS (1 g per 1 kg weight). Records as Fig. 1.

obtained in the first and second series of experiments. Reflexes from the chemoreceptors of the isolated segment of small intestine in most cases were diminished. In 8 experiments, the blood pressure reflex diminished after PAS injection; it increased in 2 experiments.

Of 10 experiments in which reaction to carbonic acid was investigated, the blood pressure reflex disappeared completely in one, was decreased in eight, and slightly increased in one.

At the same time, reflexes from the urinary bladder mechanoreceptors were not only not decreased, but (in most cases) were enhanced. Such enhancement was observed in 7 out of 10 experiments, while slight diminution was seen in 3 experiments.

Reflexes from the carotid sinus zone receptors were, as a rule, inhibited (in this case we were concerned with a complex reflex determined by stimulation of chemo- and baroreceptors in the sinus). In 4 out of 9 experiments, considerable inhibition of the blood pressure reflex was noted; less marked inhibition occurred in 5 experiments. These data are presented in Fig. 2.

It was therefore established that PAS exerted different effects on various types of interoceptive reflexes. This was expressed in the fact that PAS predominantly inhibited reflexes which arose as the result of chemoreceptor stimulation.

The fourth series of experiments was concerned with attempts to localize more precisely the action of PAS in the efferent portion of the reflex arc, and consisted of studies on the effect of this preparation on reactions elicited by direct electric stimulation of the peripheral part of the vagus, transected in the neck, and the peripheral part of the splanchnic nerve. These experiments revealed that PAS inhibited, to some extent, transmission of excitation from the vagus to the heart. The preparation did not, however, exert any consistent effect on the peripheral part of the reflex arc innervating abdominal blood vessels and the adrenal gland.

These data on the distinctive features of the PAS effect on interoceptive reflexes obtained on parenteral administration of the preparation suggested the importance of determining in what part of the central nervous system was the blocking action of PAS on reflexes from chemoreceptors effected. With this aim in view, we undertook a fifth series of experiments, in which the influence of PAS on reflexes from chemo- and mechanoreceptors was studied on 9 decerebrate animals. It was found that in experiments without anesthesia but with transection at the collicular level, PAS (0.25-1 g per 1 kg body weight) given intramuscularly, in the majority of cases inhibited the reflexes (Fig. 3). In 5 experiments in which carbonic acid was used as chemoreceptor stimulant, the blood pressure reflex was diminished in four, and enhanced in one. Stimulation of the chemoreceptors by acetylcholine was performed in 7 experiments, in six of which administration of PAS led to diminution of the reflex, and in one of which the reflex was enhanced. Under similar experimental conditions, the reflexes arising from stimulation of the urinary bladder mechanoreceptors were not inhibited by administration of PAS.

Consequently, the predominantly inhibitory effect of PAS on reflexes elicited by stimulation of chemoreceptors can be realized in decerebrate animals also.

Results of the experiments on the action of intramuscular PAS on interoceptive reflexes lead to the conclusion that the selective inhibitory effect of this preparation on reflexes from chemoreceptors is achieved by direct action of PAS on the central elements of the chemoreceptive reflex arc. The preparation can be said to exert its action by predominant inhibition of the conducting part of the internal chemical analysor.

SUMMARY

Studies of the effect of PAS on interoceptive reflexes have shown that intramuscular injections of PAS inhibit mostly reflexes elicited by stimulation of chemoreceptors. On the other hand, reflexes from mechanoreceptors were mostly enhanced. The inhibiting effect of PAS on chemoreceptors may also be elicited in the decerebrate animal.

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MICROBIOLOGY AND IMMUNITY

THE EFFECT OF BACTERIOPHAGIA ON THE GROWTH AND DEVELOPMENT OF BACTERIA

COMMUNICATION 1. ON CYTOPLASTMIC GRANULES OBSERVED IN BACTERIA DURING PHAGOCYTOSIS

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Recently, much attention has been given to the study of the morphological organization of bacteria, and to their granular, cytoplasmic elements particularly. This question is especially interesting in connection with the morphological changes occurring in bacterial cells due to bacteriophagic action.

In this article, we present the results of observations concerned with the peculiarities of the cytoplasmic granules appearing in the course of bacteriophagic lysis.

EXPERIMENTAL METHODS

Bacteriophagic lysis was observed on typical cells of a Bact. coli aerogenes culture, strain 1321.

To observe what happened to individual microbes attacked by the phage and put in conditions favorable to development, we used the method of phase-difference microscopy during the life of the cell; the preparations used in this method were prepared by the method described by V. L. Troitsky and Z. G. Pershina [4].

We used suspensions of Bact. coli aerogenes culture, strain 1321.

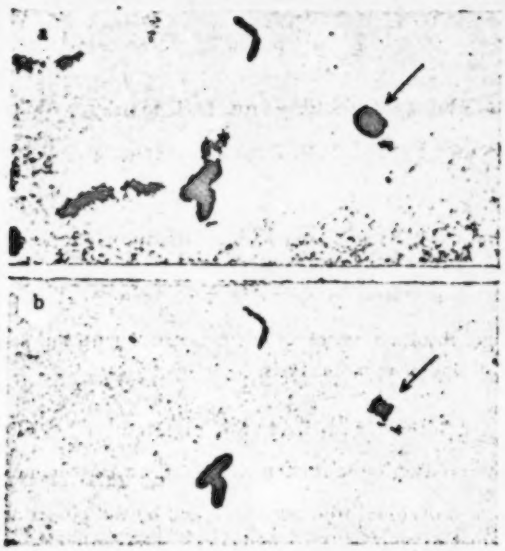
To observe what happened to individual microbes attacked by the phage and put in conditions favorable to development, we used the method of phase-contrast microscopy during the life of the cell; the preparations used in this method were prepared by the method described by V. L. Troitsky and Z. G. Pershina [4].

We used suspensions of Bact. coli aerogenes in a specific bacteriophage (titer from 10-6 to 10-7 according to Appelman), with a concentration of 1 million microbes per ml, as source material for the preparations. Instead of a heating process, we used a special incubator to maintain a stable temperature of 36°; the phase-contrast microscope with the preparation was placed in this incubator during the experiments. After finding corresponding fields of vision, the bacteria cells contained in them were designated by numbers on a chart made to scale on the field of vision in order to facilitate observation. This way of computing the results made it possible to observe several cells simultaneously, for, in most cases, the cells were lysed rather quickly.

EXPERIMENTAL RESULTS

Twelve experiments were conducted, in which we observed 183 microbes during bacteriophagic lysis. One hundred and fifty-nine of these lysed at intervals of 40 minutes–6 hours after observation began; rapid lysis was preceded by the formation of spherical, fusiform and other forms. Four cells lysed by means of a slow and even hyalinization of the protoplasm, until the original contours of the cells were lost, which occurred 8-20 hours after the experiment began. In this article, we shall not give a detailed description of the lyses and the preceding morphological changes; we shall only note that there were no cells in this group with any discernable structural differentiation in the protoplasm which might lead to the formation of granules. When the remaining 20 of the phage-attacked cells were examined, granules were found which were characterized by the following features. In 12 of these 20 cells, the appearance of the granules was connected with the hyalinization of the

protoplasm, which continued for 2-20 hours after the preparation was placed in the incubator. In some cases, the size of the bacteria did not change, and, by the end of the experiment, these cells were characterized by light protoplasm and granules. In other cases, the cells lost their contours completely due to the continuing, "thaw-like" hyalinization of the protoplasm, but the contours were replaced by isolated granules. The amount and distribution of the granules varied greatly. In 3 cells, there was 1 granule in the center or on one of the poles; in 8 cells, there was 1 granule on each of the poles; and, finally, there was 1 cell with 6 cytoplasmic granules, two on the poles, and four in different parts of the protoplasm. In the phase-contrast microscope, the granules looked like dense, rounded formation equal to or slightly smaller than the bacteria in diameter.



Zeitraffer microfilm showing phagocytosis of bacteria. Phase-contrast.
a) spherical form (arrow) of enteric bacillus before bacteriophagic lysis; b) granule (arrow) remaining after bacteriophagic "explosion" of enteric bacillus cell.

In some specimens, we also recorded protoplasm hyalinization and granule differentiation after a preliminary formation of gigantic forms (4 cells) which were 2-3 times as wide and 5-6 times as long as ordinary cells of this type of bacteria. And finally, in 4 cells, granule differentiation began with protoplasm hyalinization and was completed by the inflation or elongation of the specimens, followed by a lytic "explosion". After such a lytic reaction, the granules remained in the same place occupied by the cells before.

The preservation of the granules after the cell had lysed by instantaneous "explosion" was also observed when no previous granule differentiation had been seen in the cytoplasm. In this case, the cells only slightly lost their contrastability and, after forming a sphere or spindle, "burst," leaving granules behind them. This latter observation was recorded by a Zeitraffer microfilm in the AMS USSR department of microfilm scientific documentation (see Figure). The figure shows frames illustrating a bacterial cell of spherical shape before the "explosion," and the granule remaining after the lysis of the cells.

DISCUSSION OF RESULTS

One must take into account the literary data when evaluating the results we obtained.

In 1934, N. N. Zhukov-Verezhnikov and V. A. Friauf [1] established that the effect of a bacteriophage on typhoid fever bacillus causes formations to appear in the central portion of the cell, which the authors treated as typical nuclei in view of the fact that they contained peculiar figures of division. Other authors [3, 16] confirmed this observation.

However, the appearance of nuclear formations in the bacterial cells was apparently not the only manifestation of bacteriophagic influence.

In 1949, Boyd [8], conducting phase-contrast microscopy of enteric bacteria cells attacked by a bacteriophage, discovered peculiar, dense zones on the poles of the bacteria which he believed to be protoplasmic thickenings caused by the division of the phage in the central portion of the cells. Heden [13] observed similar pictures in phase-contrast and ultraviolet microscopes. In his opinion, the protuberant polar granules were accumulations of phagic corpuscles.

Later on, these granules, located at the poles or in other portions of the cytoplasm, began to be considered mitochondria. A similar opinion had existed earlier [10, 15], but was not confirmed until later by works of researchers who had ceased to indentify these elements with nucleoids (nuclei) in the form of single, but complex, organelles [6, 7].

The chemical properties of bacterial mitochondria were defined at the same time [20].

These granules have now been discovered in many bacteria and are considered to be definite cytoplasmic structural elements, which are the centers of coordinated enzymatic oxidizing-reducing reactions in the cytoplasm [5, 9, 11, 12, 17, 18, 19, 21, 23, 24, 25].

We propose that the granules which we found, like the granules found earlier in sound-treated microbes of a different type [2], are analogous to mitochondria, since there are indications that mitochondria of bacteria are more resistant to the bacteriophage, antibiotics and to a series of other influences [12, 24]. The nuclear substance evidently disappears in phagocytosis. Of course, this proposition must be experimentally confirmed. However, our data show that the resistance of the granules to the phage is of a strictly individual character. Only 20, or about 11%, of the 183 enteric bacillus cells contained the cytoplasmic granules. It is at present difficult to explain the reasons for this phenomenon. One can only suggest that it is explained by the activity of the phage corpuscles adsorbed into the surface of the cells as well as by the individual properties of the bacterial cells. The heterogenicity among the lytic reactions of the microbe and the difference degrees of heterogenization of their protoplasm in the differentiation and liberation of granules must be regarded in the same way.

From our observations and from the literary data, one can conclude that the theory regarding bacteriophagic lysis as a phenomenon ending in the complete destruction of bacterial morphological organization must be more accurately defined, since, in a definite number of cases, some cytoplasmic elements survive.

We are not concerned in this article with the fact that filtrable forms of bacteria remain capable of life after lysis, as that is a question which needs further study.

SUMMARY

In vivo dynamics of phagocytosis in 183 Bact. coli aerogenes strain 1321 were studied by phase-contrast microscopy. The formation of solid cytoplasmic granules was noted in 20 cells, and in some cases the granules could be seen after lysis of the bacterial cells. Microfilms revealed granules after lysis even in cells in which no protoplasmic differentiation was noted before "explosion." It is probable that these granules are identical with mitochondria, and possess an individual resistance to the phage.

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THE EFFECT OF SHORTWAVE LENGTH ULTRAVIOLET RAYS ON THE INFECTIOSITY AND ANTIGENIC PROPERTIES OF THE VIRUSES OF RUSSIAN TICK-BORNE AND JAPANESE ENCEPHALITIS

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We conducted experiments to investigate the effect of ultraviolet rays on the infectiosity and antigenic properties of tick-borne encephalitis.

As is known from the data in the literature, there are different physical and chemical agents in use to eliminate the infectiosity of neuroviruses which preserve the antigenic properties of the viruses.

One of the most widely used inactivators is formalin, which is used to prepare various neurovirus vaccines. In the great majority of cases, however, the use of formalin in a 1:500 concentration causes the titer of antigens to decrease and their anticomplementicity to increase [1, 2, 4, 5].

The possibility of eliminating the infectiousness of viruses while retaining their antigenic properties is mentioned [3, 6] in individual works studying neurovirus inactivation caused by ultraviolet rays 2537 A long.

However, the data given in these works on the time of inactivation are contradictory, and there is no exact data on the relation between the antigenic and infectious properties, which data is necessary to obtain highly active antigens from a dose of ultraviolet radiation. There is almost no data on the virus of tick-borne encephalitis.

EXPERIMENTAL METHODS

For radiation, we used: a BK bactericidal lamp giving ultraviolet radiation, 85% of which fell within shortwave length ultraviolet rays (SUV) with a wave length of 2, 597 A, and a portable quartz mercury lamp (PQM-4) radiating the whole spectrum of ultraviolet rays, with all the shortwave length ultraviolet rays intercepted by an ultraviolet-transmitting glass filter, so that the maximum radiation consisted of longwave length ultraviolet rays (LUV) with a wave length of 2,970-3,020 A.

Virus-containing suspensions of different concentrations (1 and 10%), from the brain tissue of mouse and chicken embryos infected with the virus of tick-borne or Japanese encephalitis, prepared in a physiological solution of sodium chloride, were subjected to radiation. In a series of experiments, the allantoic fluid from chicken embryos infected with the virus of tick-borne encephalitis was used.

The virus-containing material was centrifuged for 30 minutes at 3,000 revolutions per minute and put in a Petri dish or byuks* so that the suspension to be irradiated was 1 mm thick.

The lamp burners were placed above the suspension to be radiated so that the radiant energy fell at right

* Transliterated.

angles to the suspension. Radiation intensity was 200-210 microwatts per 1 cm^2 in all of the experiments. Shortwave length radiation was done at a distance of 25 cm, longwave, at a distance of 17 cm. The radiation lasted 5, 10, 15, 25, 30 and 40 minutes.

The suspension's loss of infectiosity was determined by intracerebrally injecting it simultaneously into 5-6 mice in a dose of 0.03 ml. If there were no clinical signs of sickness in the primarily infected mice, three of them were killed, and their brains passaged to fresh mice.

EXPERIMENTAL RESULTS

We obtained the following results from the experiments testing the inactivation of the tick-borne encephalitis ("Sofyin" strain) virus contained in the various tissue suspensions which were radiated with SUV rays 200-210 microwatts per cm^2 in intensity.

The virus contained in the 10% suspension of mouse brain tissue was completely inactivated after 15-minute or longer radiation. The mice whose brains were infected with the irradiated suspensions did not get sick, and the virus was not evident in the first passage to the mice (Table 1).

TABLE 1

Effect of SUV Radiation (200-210 microwatts per 1 cm^2) on the Infectiosity (Inactivation) of the Virus of Russian Tick-Borne Encephalitis

Material	Radiation time (in min)	Inactivation control				Inactivation results
		Number of mice				
		Infected	Died	Passaged	Died	
Centrifugate of 10% suspension from brain of infected mice	15	5	0	5	0	+
	20	5	0	5	0	+
	30	5	0	5	0	+
	40	5	0	5	0	+
The same	Not irradiated	5	5	×	×	—
Centrifugate of 10% suspension from brain of infected chicken embryos	5	6	6	×	×	—
	10	6	6	×	×	—
	15	6	2	×	×	±
	20	6	0	6	0	+
	25	6	0	6	0	+
	30	6	0	6	0	+
The same	Not irradiated	6	6	×	×	—

Symbols: + positive, — negative, x not investigated

When the 10% suspension of chicken embryo brain tissue with a virus titer of 10^{-6} was irradiated, complete inactivation of the tick-borne encephalitis virus was obtained after 20-, 25- and 30 minute radiation. When the 10% suspension was irradiated for 15 minutes, only partial inactivation of the virus was obtained, and with 5- and 10- minute radiation, the virus was not inactivated (Table 1).

The virus contained in the undiluted allantoic fluid of the chicken embryos was partially inactivated after 20- and 40- minute irradiation, but not inactivated by 10-minute radiation (Table 2).

Irradiation of the allantoic fluid in a dilution of 1:5, however, caused complete inactivation of the virus after 10-, 20- and 40-minute exposures (Table 2).

The next experiments were done with the Japanese encephalitis virus, contained in a 1% suspension of mouse brain tissue. Shortwave length (SUV) and longwave length (LUV) rays were used for the radiation (Table 3).

TABLE 2

The Effect of SUV rays (200-210 microwatts per 1 cm^2) on the Infectiosity of the Tick-borne Encephalitis Virus Contained in Allantoic Fluid

Material	Radiation time (in min)	Inactivation control				Inactivation result
		Number of mice				
		Infected	Died	Passaged	Died	
Undiluted allantoic fluid	10	5	5	×	×	—
	20	5	2	×	×	±
	40	5	1	×	×	±
Allantoic fluid diluted 1:5	10	5	0	5	0	+
	20	5	0	5	0	+
	40	5	0	5	0	+
The same	Not irradiated	5	5	—	—	

Note: Symbols mean the same as in Table 1.

The experiments showed that the inactivating effect of the SUV rays extends to the virus of Japanese encephalitis. Irradiation of the 1% suspension from the brain tissue of infected mice caused inactivation of the virus after 5, 10 and 15 minutes of radiation.

TABLE 3

The Effect of Radiation with Ultraviolet Rays of Various Wave Lengths of the Infectiosity of the Japanese Encephalitis Virus

Material	Rays	Radiation time (in min)	Inactivation control				Inactivation results
			Number of mice				
			Infected	Died	Passaged	Died	
Centrifugate of 1% suspension from brain of infected mice	SUV	5	4	0	4	0	+
		10	4	0	4	0	+
		15	4	0	4	0	+
	LUV	5	4	4	×	×	—
		10	4	4	×	×	—
		20	4	4	×	×	—
		30	4	4	×	×	—
The same		Not irradiated	4	4	×	×	—

Note: Symbols mean the same as in Table 1.

Parallel experiments were also conducted, irradiating a similar suspension with LUV rays of the same radiant energy intensity — 200-210 microwatts per 1 cm^2; these rays did not inactivate the virus after 5, 10, 20 or 30 minutes of radiation (Table 3).

Therefore, irradiation with shortwave length ultraviolet rays causes inactivation of the tick-borne and Japanese encephalitis viruses, while irradiation with longwave length ultraviolet rays of the same intensity does not. With radiation by shortwave length ultraviolet rays of unvarying intensity, inactivation depends on the duration of the irradiation, i.e., on the dose of radiant energy, and also on the concentration of the tissue components in the virus-containing material undergoing irradiation.

The antigenic properties of the virus were tested by a complement fixation reaction with specific (experimental) or convalescent serums.

Allantoic fluid was used in the experiment without additional processing, and a thermolysed antigen was

prepared from a 10% suspension of chicken embryo brain tissue. The complement fixation reaction was done by the method of cold agglutination.

To find out how antigenic properties change in a virus irradiated with SUV rays but not inactivated, we examined undiluted, virus-containing allantoic fluid which had been irradiated for 5, 10 and 20 minutes. The results of the reaction showed that the antigenic properties of the tick-borne encephalitis virus do not change when the infectiosity of the virus is preserved, but change slightly due to 20-minute irradiation, which causes partial inactivation of the virus (Table 4).

TABLE 4

Complement Fixation Reaction with Allantoic Antigens of the Tick-Borne Encephalitis Virus Irradiated by SUV rays

Complement dose	Radiation time (in min.)	Antigens	Serum and dilution									Antigen control
			Specific No. 1 (exptl.)			Specific No. 2 (experimental)			Normal			
			1:4	1:8	1:16	1:4	1:8	1:16	1:4	1:8	1:16	
2.0	5	Specific	4	3	2	2	2	—	—	—	—	—
	10	"	4	4	3	3	3	—	—	—	—	—
	20	"	3	3	2	—	—	—	—	—	—	—
	0	"	4	4	3	2	2	+	—	—	—	—
	0	Normal allantois	—	—	—	—	—	—	—	—	—	—
	0	Control serum	—	—	—	—	—	—	—	—	—	×

Symbols: specific antigen — allantoic fluid of chicken embryos infected by virus of tick-borne encephalitis; 4, 3, 2 +) degree of hemolysis inhibition; x) complement fixation reaction not studied; —)complete hemolysis; 0) not irradiated.

TABLE 5

Antigens of Tick-Borne Encephalitis Inactivated by Ultraviolet Rays Tested in Complement Fixation Reaction

Radiation time (in minutes)	Antigen in dilution	Serum in dilution of 1:8										Antigen control		
		Specific					Normal							
		1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40	1:80	1:160	1:10	1:20	1:40
5	Specific 10%	4	4	4	2	—	—	—	—	—	—	—	—	—
10	The same	4	4	4	+	—	—	—	—	—	—	—	—	—
15	"	4	4	4	±	—	—	—	—	—	—	—	—	—
20	"	4	4	3	±	—	—	—	—	—	—	—	—	—
30	"	4	4	2	±	—	—	—	—	—	—	—	—	—
0	"	4	4	4	2	—	—	—	—	—	—	—	—	—
0	Normal	—	—	—	—	—	—	—	—	—	—	—	—	—
0	Control serum	—	—	—	—	—	—	—	—	—	—	—	—	—

Symbols: Same as in Table 4.

In order to more exactly determine how much the antigenic properties of the virus decreased in relation to the degree of virus inactivation, we conducted further experiments with a 10% suspension of chicken embryo brain tissue, which was irradiated 5, 10, 15, 20 and 30 minutes and then used to prepared thermolysed antigens.

The control antigens were prepared from a nonirradiated virus-containing suspension (positive control antigen) and from a 10% brain tissue suspension from uninfected chicken embryos (normal antigen).

These antigens were titrated in a complement fixation reaction with a specific or a normal serum, used in a dilution of 1:8. The results obtained are shown in Table 5.

The results of these experiments showed that the titers in the complement fixation reaction of the antigens prepared from the completely inactivated suspension of the tick-borne encephalitis virus (20-30 minute radiation) were twice as low (1:40) as those of the noninactivated antigens prepared from the same initial suspension (titer of nonirradiated antigen – 1:80).

Therefore, when the tick-borne encephalitis virus is inactivated by SUV rays, the virus retains its antigenic (complement agglutinizing) properties even after the complete loss of its infectiosity (Table 1 and 5). In this case the titer of antigens is twice as low as the titers of natural antigens prepared from the same initial suspension (Table 5). However, increasing the SUV rays to more than the minimal dose (increasing radiation time), i.e., a stronger dose than is needed to completely eliminate the infectiosity of the virus, causes a greater decrease in the antigenic (complement agglutinizing) properties of the tick-borne encephalitis virus.

SUMMARY

Irradiation for 5-20 minutes by shortwave lengths of 2597 A of 1-10% brain suspensions infected with Russian tick-borne or Japanese encephalitis virus resulted in complete inactivation of the virus. The same suspensions irradiated by longwave lengths of 2970-3020 A retained their infectiosity even after 30 minutes of irradiation. In complement fixation tests, virus antigens inactivated by shortwave lengths irradiation reacted positively with specific serum.

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ON THE PROBLEM OF FINDING TISSUE ANTIGENS

COMMUNICATION I. THE RELATIONSHIP BETWEEN THE SENSITIZING AND SHOCK DOSES OF ANTIGENS IN ANAPHYLAXIS

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The representatives of many branches of biology and medicine are interested in the characteristics of the antigenic composition of animal and human normal and pathologic tissues. Such immunological studies are useful in studying the processes of tissue metamorphasis and development in ontogenesis; they are helpful in determining the character of various pathological changes in tissues, and they have a place in comparative and evolutional zoology. The investigation of this question is especially important to the study of the problems involved in the tumorous process. However, it is admittedly very difficult, technically, to examine the antigenic composition of tissue, since this involves finding and differentiating the different individual components contained in a complicated antigenic complex. Therefore, highly sensitive methods of research must be used. A method permitting even the smallest inexactitude increases the possibility of "experimental error", thus decreasing the conclusiveness of the experimental results. Therefore, a knowledge and comparative evaluation of all the immunological methods available to research is of paramount importance to this question as a whole.

Experimental analysis of this question is the purpose of these articles. We were primarily interested in the immunological methods used in the study of tumors. It seemed more expedient, however, to temporarily shelve specific oncological problems (as well as the specific problems of other branches of science) and to consider the subject on a general immunological basis first. The first step, then, was to determine the conditions best suited to the disclosure of the individual components of an antigenic complex, depending on the quantitative relationships existing between these components, and to determine the research methods best suited to this purpose. The purpose of this work was the investigation of this question.

EXPERIMENTAL METHODS

A double set of model experiments were conducted, with "strong" or "weak" antigens, depending on the degree to which the physical immunological reaction effected by these antigens was usually expressed. We used some heterogeneous serum as the "strong" antigen and nucleoproteins from normal tissues as the "weak" antigen. Depending on the experimental conditions, we injected the antigens either separately or mixed in various ratios. We used the anaphylactic reaction and serological methods to disclose the antigens.

The nucleoprotein preparations were prepared by the method used in research to disclose specific tumorous antigens [1]. The tissue was ground up; to the resulting mass, a triple quantity (in relation to the tissue weight) of a physiological solution and then a 1% solution of NaOH to pH=9 were added; the material was then kept at a temperature of 4° until the following day. The fluid obtained after 2-minute centrifugation at 3,500 revolutions per minute was acidified with a 10% solution of acetic acid to pH=6, the precipitate was separated by centrifugation, and the remaining fluid was acidified to pH=4.5. The precipitate was dissolved in distilled water and alkalized with a 1% solution of caustic alkali to pH=7.2. Merthiolate in a concentration of 1:10,000 was added

to the resulting solutions of nucleoprotein. The preparation was kept at a temperature of 4°. The protein content was determined by Kjeldahl's method (microdetermination). The relationship between the sensitizing and shock dose amounts had to be determined in order to find under what conditions the anaphylactic reaction could be used to disclose an individual component constituting only a small part of the antigenic complex.

Besides data on the well-known fact that a smaller dose of antigen is necessary for sensitization than is needed for the reacting injection, the literature also contains some information concerning the quantitative relationships between the sensitizing and shock doses. In the experiments of Doerr and Burger [2, 3], for example, the minimal sensitizing dose of serous euglobulin (horse serum) was 0.0004 mg. However, 0.1 mg of the same antigen had to be injected in order to produce shock, i.e., 250 times more. Similar relationships (0.0039 and 0.1 mg) are also mentioned in connection with the albumin group, salted out at 56-66% saturation with ammonium sulfate. The minimal sensitizing dose for an albumin group precipitating at 66-99% saturation with ammonium sulfate was 0.08 mg, the shock dose, 1.9 mg.

In our experiments, separate groups of guinea pigs were sensitized to horse serum, which was injected intravenously in doses of from 0.025 to 0.0001 ml per 100 g of weight, (from 0.024 to 2.4 mg of protein) into guinea pigs weighing 300 g each.

The results of the experiments are given in the table.

EXPERIMENTAL RESULTS

The shock dose (see Table) was equal to the sensitizing dose only when sensitization was done with a comparatively large amount of serum: 0.01 ml per 100 g of weight, or 2.4 mg of protein per 300 g guinea pig.

Relationship Between Sensitizing and Shock Doses of the Antigen (Horse Serum)

Reacting dose in ml per 100 g of weight	Sensitizing dose in ml per 100 g of weight				
	0.025	0.01	0.0025	0.001	0.0001
0.1					Died
0.05 - 0.05		Died		Died	
0.03					
0.01			Died		
0.005					
0.0025 – 0.002					
0.001					
0.0001					

Symbols: +++ pronounced anaphylactic shock with acute asphyxiation, loss of ability to stand, convulsions; ++ clear anaphylactic reaction with signs of bronchial spasm expressed (convulsive cough); + anaphylactic signs in the form of scratching, sneezing, exhaustion without distinct signs of bronchial spasm; – no signs of anaphylactic shock.

If the sensitizing dose was decreased to 0.6 mg of protein per 300 g guinea pig, then a reacting dose of the same or even a double amount of the antigen did not cause an expressed reaction; anaphylactic shock was only observed (a lethal shock, in one case) when 0.01 ml of the antigen per 100 g of weight was injected, i.e., an amount 4 times greater than the sensitizing dose.

When the sensitizing dose was still smaller (0.001 per 100 g of animal weight), 30-50 times the amount of the sensitizing dose of antigens had to be injected in order to obtain a shock reaction, although a clear anaphylactic reaction (++) was observed with a reacting dose consisting of 5-10 times the sensitizing dose, i.e., 0.005-0.01 ml of serum per 100 g of weight. With a still smaller sensitizing dose — 0.0001 ml per 100 g of weight, the injection of a dose 100 times larger caused no reaction at all; only when the antigen was injected in a dose 500 times that of the sensitizing injection was a clear (approaching shock) reaction observed, while the reacting dose had to be increased to 1,000 times the sensitizing dose in order to cause lethal shock.

In another experiment, the guinea pigs were sensitized to nucleoprotein from a horse's liver (weak antigen), with a protein content of 20 mg per 1 ml.

To sensitize 300 g guinea pigs, 0.02 ml (0.04 mg of protein) was injected intraperitoneally. The reacting injection was done intravenously after 3 weeks, in doses of from 0.25 to 1 ml. Both of the guinea pigs injected with 1 ml of the preparation died. Two of the 3 guinea pigs injected with 0.5 ml died; shock was deferred in one of them, and it did not die until 12 hours after the reacting injection. A pronounced anaphylactic reaction was observed in the third guinea pig. When the amount of antigen injected in the reacting injection was still further decreased, the animals did not die; both of the guinea pigs injected with the 0.25 ml reacting dose manifested a clear anaphylactic reaction (++). Therefore, it was also necessary to increase the reacting dose to several times the amount of the sensitizing dose in order to obtain a definite result when the sensitization had been done with a small dose of weak antigen.

The known method used to find the differences in the antigenic properties of tumorous and normal tissue (by means of the anaphylactic reaction) is to sensitize the animal and then give a reacting dose consisting of so-called tumorous preparations, of which the actual tumor component constitutes only a small part. Even with a rather large sensitizing dose of the preparation (5-10 mg), the sensitizing dose of the tumor component itself is usually small. One should therefore take into account the data presented regarding the relationships between sensitizing and shock doses, since they show when one can conclude an observed anaphylactic reaction to be caused by the tumorous antigen and when one must refrain from such a conclusion.

SUMMARY

The relationship between the sensitizing and booster (shock) dose of antigens of different intensity (such as serum proteins and horse liver) was studied in experiments on guinea pigs. General interrelationships were established, which was manifested in the fact that the booster dose had to be increased, according to the gradual decrease of the sensitizing dose of the antigen.

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A COMPARISON OF THE PROCESSES OF FORMATION OF ANTIBODIES AND NON SPECIFIC PROTEINS IN ANIMALS

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In the literature data are often encountered which attest that the assimilation of protein in the body can follow various pathways. Breakdown of introduced proteins is not essential for their utilization. Assimilation of proteins in the body can evidently take place at various stages of protein breakdown and during synthesis of protein of organs and tissues structural units of various sizes may be incorporated [11, 10, 4, 1].

The question of what specific protein antibodies are synthesized from in the body is an interesting one. At the present time there hardly exists any doubt that serum proteins do not serve as direct precursors of antibodies [2, 8].

However, whether antibodies utilize for their formation amino acids only, as several authors propose [8], or appear as derivatives of certain intermediate proteins formed in the body, has thus far not been ascertained.

The fact, discovered by Schoenheimer and coworkers [9], that like other serum and tissue proteins, antibodies take part in metabolic reactions and utilize dietary nitrogen, allows one to suppose that the pathway of antibody synthesis does not differ essentially from the pathway of synethsis of any non specific protein.

At the same time, the formation of antibodies in the body differs in the peculiar dynamics of their synthesis, and also in the fact that a protein is being formed with a sharply defined specificity.

In a previous paper we called attention to the fact that the sharp acceleration of production of specific antibody protein which follows revaccination is accompanied by an activation of the synthesis of non specific serum proteins [3].

By comparing the processes of formation of antibody and ordinary serum proteins, we expected to elucidate to a significant degree the question whether antibodies are synthesized only from amino acids as structural units, or whether for their formation, proteins and peptides may be utilized directly without preliminary breakdown into amino acids, as the synthesis of tissue proteins proceeds, which was shown by a series of the above mentioned investigators. Along with this we attempted to ascertain whether there is any connection between the capacity to form antibodies, i.e., the extent of synthesis of specific proteins and the extent of synthesis of non specific proteins, particularly serum proteins.

The investigations were carried out on various groups of immune rabbits, which along with methionine labelled with S^{35}, were injected with labelled liver and serum proteins. The extent of synthesis was estimated from the radioactive amino acid content of the antibody and serum proteins.

METHODS

The proteins of a tissue homogenate and of serum were assayed for radioactive S^{35} methionine by the method described in a previous paper [1].

We immunized the rabbits with typhoid vaccine.

We carried out the experiment on 3 groups of immunized rabbits. Into animals of the first group we injected S^{35} methionine intraperitoneally according to the calculation of 1 g of weight from 4,000 to 30,000 impulses. Similarly we injected rabbits of the second group intraperitoneally with S^{35} methionine labelled liver homogenate (from 1500 to 10,000 impulses per 1 g in a volume of 25 cc), and injected the rabbits of the third group with labelled serum (from 500 to 4000 impulses per 1 g in a volume of 10 – 15 cc).

In order to equalize the period of absorption of the injected materials, as much as possible, we divided the dose of serum into two parts and injected it on 2 successive days; the liver homogenate was injected as a single dose.

We took blood from all rabbits on the 1st, 3rd, 5th, 8th and 12th day after simultaneous injection of the antigen and labelled material. We isolated the antibodies of the serum by precipitation with "diagnosticum" on the 3rd, 5th, 8th and 12th day.

After the introduction of the antibodies we precipitated the serum proteins with trichloracetic acid, and washed free of radioactivity in the usual way; we washed the antibody 5 times with cold physiological saline. Then we dried this and other samples with ether and ground them to a powder. We weighed out samples of 2 mg. We counted the radioactivity, in the antibody and the serum proteins, on aluminum disks with an end-window counter. The squared deviations of the individual determinations did not exceed 5%.

On the 3rd, 5th, 8th and 12th day, instead of a count of the radioactivity of the antibodies, we determined their titer.

In order to find out whether parenterally injected proteins could be utilized in the formation of antibodies without preliminary breakdown into amino acids, we attempted to apply the marker dilution method [7,1,5]. Therefore each of the three groups of rabbits was subdivided into two subgroups. Rabbits of the first subgroup received only the appropriate labelled substrate, but the second subgroup received in addition "ballast" unlabelled methionine.

We considered that an absence of dilution of the radioactivity of the proteins in the rabbits of the second subgroup would show that the incorporation of parenterally injected proteins into the composition of the organs and tissues takes place without preliminary breakdown into amino acids. Dilution of the radioactive marker in the proteins of the organs of the animals would indicate preliminary, profound breakdown of the injected proteins to amino acids and subsequent partial resynthesis with utilization of radioactive and ballast methionine.

However, since the determination of the radioactivity of antibodies and serum proteins was carried out, basically, many hours after the injection of the methionine, during which time it quickly left the body, the method of dilution of the marker proved poorly suited for these investigations (see Table). In addition, the non-uniformity of the results which were obtained in carrying out these experiments is evidently explained by the dependence of the extent of protein synthesis on the individual reaction of the rabbit to the injected antigen.

RESULTS

In the table are presented the average values of the radioactivity content of serum proteins and antibody of rabbits of all three series with and without injection of ballast labelled methionine.

When the data presented in the table and in Fig. 1 are examined one's attention is called to the fact that following the injection of tagged methionine there is observed a low radioactivity content in the antibody and serum proteins. The proteins of rabbits injected with tagged liver homogenate and serum were characterized by a significantly higher radioactivity and it is noted that the antibody scores highest following the injection of the rabbits with tagged liver homogenate, while the serum proteins score highest following injection of serum. Furthermore, as in seen from the table, in all the experiments the same effect was observed – the radioactivity content of the antibody and serum proteins was significantly higher if the radioactive isotope was introduced in the form of tagged protein, rather than in the form of amino acids. This indicates that the process of synthesis of both the non-specific and specific proteins utilizes not only amino acids but also larger structural units of the protein, for if it utilized only amino acids injection of them would caused antibody and serum proteins to show a higher degree of labelling than would the injection of labelled protein.

In spite of the fact that the method of marker dilution, under the conditions of our experiments, did not

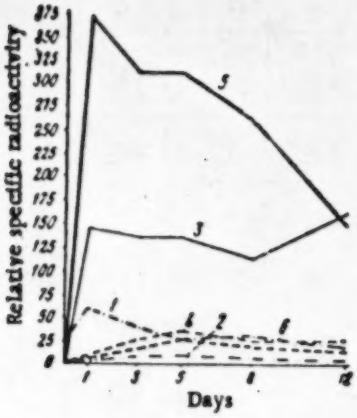

Fig. 1. Average values of radioactivity of antibodies and serum proteins after injection of labelled methionine, liver homogenate, and serum.
1) Serum Proteins + methionine (16 rabbits); 2) Antibodies + methionine; 3) Serum proteins + liver homogenate (14 rabbits); 4) Antibodies + liver homogenate; 5) Serum proteins + labelled serum (6 rabbits); 6) Antibodies + labelled serum.

give the expected results, the data obtained allow the conclusion to be drawn that antibody is not synthesized from amino acids alone; for their formation larger fragments may be utilized, possibly even whole protein molecules.

As was mentioned above, in all rabbits injected with antigen and radioactive material, the course of inclusion of the marker in the antibody is reflected in the curve of increasing titers. In addition, in the rabbits with moderate antibody titers low radioactivity characterized not only the antibody but also the serum proteins. In Fig. 2 there are presented the values for radioactivity content of antibody and serum proteins in rabbits with titers to 1:800 and in rabbits with titers from 1:1600.

On comparing the increase in antibody titer and the content of radioisotope in the non-specific serum proteins after injection of labelled proteins, the relation between the degree of immunogenesis and the degree of labelling of the non-specific proteins is clearly evident. An analogous relation is evident also following the injection of labelled amino acids into rabbits of various immunological reactivity (Fig. 2 B), however in this case it is less clearly expressed, which is perhaps explained by the low radioactivity of the antibodies and proteins, which makes the differences less noticeable.

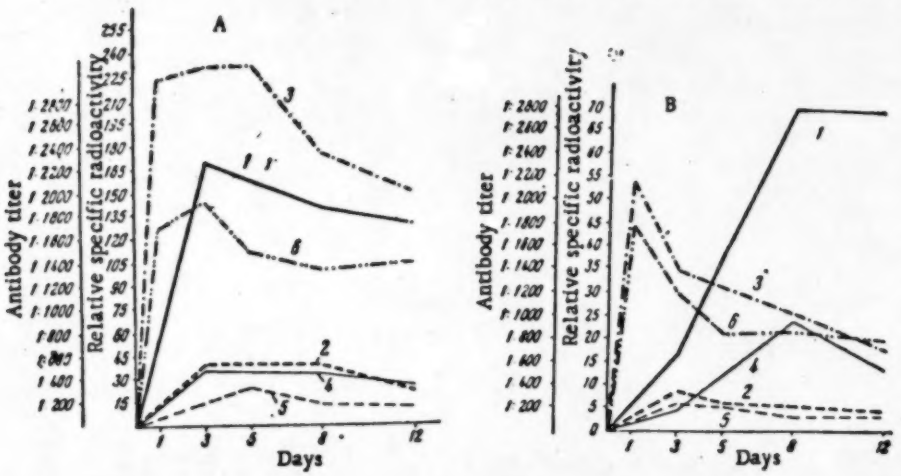

Fig. 2. Relation between antibody titers and radioactivity values of serum proteins and antibodies.
A. Injection of labelled proteins (Group I) 6 rabbits, titers from 1:1600; group II) 4 rabbits, titers to 1:800); B – Injection of labelled amino acid (group I – 7 rabbits, titers from 1:1600, group II) 3 rabbits, titers to 1:800).
1) Antibody titers of group I; 2) radioactivity of antibody of group I; 3) radioactivity of serum proteins of group 1; 4) antibody titer of group II; 5) radioactivity of antibodies of group II; 6) radioactivity of serum proteins of group II.

Radioactivity of Antibody and Serum Proteins Following Injection of S^{35} Methionine, Labelled Liver Homogenates and Labelled Serum, Alone and with "Ballast" Methionine

Material	No. of experiment	Radioactivity of injected material (imp/g)	Rel. Specific activity without ballast methionine										Rel. specific activity with ballast methionine									
			antibody					serum proteins					Antibody					serum proteins				
			no. of animals	3rd day	5th day	8th day	12th day	24 hours	3rd day	5th day	8th day	12th day	no. of animals	3rd day	5th day	8th day	12th day	24 hours	3rd day	5th day	8th day	12th day
S^{35} Methionine	I	10 000	2	24.5	20	8	6	120	85	50	55	44	2	15	13	5	7	110	62	36	31	22
	II	17 000	2	4.5	4	3.5	1	45	27	30	20	16	2	5	2	3.5	2.5	57	34	32	28	20
	III	30 000	2	2.5	2	2	1 5	30	18	13	20	12	2	2 5	2	2	2	31	20	10	12	9
	IV	4 000	2	11	12	14	11	39	35	45	30	14	2	8	18	14	8	52	36	42	33	20
		Average	8	10.5	9.5	7	5	58	41	34	31	22	8	7.5	9	6	5	62	38	30	26	18
Labelled liver homogenate	I	1 500	1	6.5	—	10	—	55	65	35	30	—	1	8	—	5	—	53	55	45	40	—
	II	2 900	2	23	—	—	—	104	110	65	80	—	2	20	—	—	—	150	115	160	160	—
	III	3 175	2	32	40	45	42	217	207	220	132	180	2	27	21	24	20	195	145	142	150	145
	IV	7 634	2	35	39	34	13	177	212	202	180	162	2	42	40	36	24	202	207	207	162	137
		Average	7	24	39	29	27	138	148	131	105	171	7	24	30	22	22	150	131	138	128	141
Labelled serum	I	500	1	25	—	25	25	515	385	—	—	125	1	0	—	10	10	470	280	—	—	170
	II	1 855	1	—	33	30	32	—	—	395	330	180	1	—	30	35	20	—	—	355	350	170
	III	3 110	1	16	28	12	12	195	275	200	150	100	1	25	25	26	13	310	310	305	210	150
		Average	3	20	30	22	23	355	330	297	240	135	3	13	27	23	14	390	295	330	280	160

The immunological reactivity of an animal is evidently closely connected with its individual metabolic rate. F. Dixon and coauthors, studying the survival of antibodies in various species of animals, came to the conclusion that the rapidity of γ-globulin metabolism depends on the rate of metabolism in the species in question [6]. Our data also allow one to conclude that the metabolic rate of an animal may determine its capacity for immunogenesis.

The ability of an organism to make antibody is evidently closely connected with the general level of synthesis of non specific proteins.

In previous studies we remarked [3] that at the time of revaccination the synthesis of non-specific serum proteins is activated. At the present time there are no tests which can be used to determine the immunological reactivity of an organism before the introduction of the antigen.

The connection between the extent of synthesis of non specific protein in an organism and its capacity to produce antibody, which appears from our experiments, allows one to suppose that the determination by isotopic markers, of the extent of inclusion of structural units of protein in serum proteins may, in more refined studies of the question, serve as an indicator of the immunological reactivity of the subject.

SUMMARY

The utilization of liver and serum proteins and amino acid (S^{35} methionine) in the process of formation of specific (antibodies) and non specific proteins in rabbits was studied.

Thirty-six immunized rabbits were divided into 3 groups. Into the first group S^{35} tagged liver homogenate was introduced simultaneously with the antigen, into the second group S^{35} tagged serumand into the third group S^{35} tagged amino acid.

By comparing the amount and degree of incorporation of S^{35} in the antibodies and serum proteins it was established that in all groups of animals both proteins and protein fragments are utilized in the synthesis of antibodies and non specific serum proteins. Proteins are evidently utilized with greater ease and rapidity. The degree of antibody production is closely connected with extent of synthesis of non specific proteins in the individual animal.

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THE INFLUENCE OF ANESTHESIA OF THE SITE OF INTRODUCTION OF VACCINE ON THE DEVELOPMENT OF VACCINE REACTION

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The question of the influence of anesthesia of the site of introduction of antigen on immunological reactivity has been little studied. The result of such an influence evidently depends on the conditions of the experiment. Thus, the infiltration of 30 ml of 0.5 % novocaine solution into the muscle of a rabbit caused a diminution of antitoxin formation following repeated immunization with tetanus toxoid [3]. In agreement with this, in a communication of E. V. Konovalov [2], when rabbits were injected interarticularly with dysentery bacteria, anesthesia cut antibody production in half.

On the other hand, when the anesthetic acts more weakly (moderate amount of novocaine, vaccine not introduced into a closed space), anesthesia of the area of introduction of antigen increases antibody formation (to tetanus toxoid) 7 – 9 times [1]. Intravenous injection of a moderate amount of novocaine into human beings (0.5 – 1 mg per kg weight) increases the complement titer of the serum [4].

TABLE 1

Influence of Novocaine on Development of General Inoculation Reaction Following Vaccination with Polyvalent Vaccine

Group	Method of immunization	No. persons vaccinated		Number of reactions						Av. time of development of reaction (hrs)
				strong		moderate		weak		
		actual	%	actual	%	actual	%	actual	%	
First	Control	200	100	6	3	22	11	49	24.5	6
Second	with novocaine	1500	100	16	1.06	73	4.86	165	11	9

TABLE 2

Influence of Novocaine on Development of Local Inoculation Reaction Following Vaccination with Polyvalent Vaccine

Group	Method of immunization	No. persons vaccinated		Number of reactions						Av. time of development of reaction (in hrs)
				strong		moderate		weak		
		actual	%	actual	%	actual	%	actual	%	
First	Control	200	100	3	1.5	6	3	74	37	24
Second	with novocaine	1500	100	9	0.6	26	1.73	285	19	36

TABLE 3

Agglutination Reactions of Rabbits Against Typhoid Antigen

No. of rabbit	Group	Weight (grams)	Sex	Time of test bleeding: Before immunization					After immunization: 14 days					21 days					28 days					40 days					60 days				
									titer of serum																							
				1 : 40	1 : 80	1 : 160	1 : 320	control	1 : 40	1 : 80	1 : 160	1 : 320	control	1 : 40	1 : 80	1 : 160	1 : 320	control	1 : 40	1 : 80	1 : 160	1 : 320	control	1 : 40	1 : 80	1 : 160	1 : 320	control	1 : 40	1 : 80	1 : 160	1 : 320	control
1	4% novo-caine	1960	Male	—	—	—	—	—	±+	++	+	—	—	±+	++	—	—	—	++	++	—	—	—	++	—	—	—	—	—	—	—	—
2		1840	female	—	—	—	—	—	++++	++++	++	++	—	+++	++	++	—	—	+++	+++	++	+	—	+++	++	+	—	—	++	±+	±+	±	—
3		2000	male	—	—	—	—	—	++	+	—	—	—	+++	++	++	—	—	+±+	++	+	—	—	++	++	—	—	—	+++	+++	+	±	—
4		2056	male	—	—	—	—	—	++++	+++	++	—	—	±+	++	+	—	—	+++	++	+	—	—	++	++	—	—	—	+	—	—	—	—
5		1570	male	—	—	—	—	—	++++	++++	+++	+++	—	++	++	++	—	—	+++	++	+	—	—	+++	++	—	—	—	±	±	—	—	—
6	1% novo-caine	1950	male	—	—	—	—	—	±++	±±	++	—	—	++++	++++	+++	+	—	++	++	—	—	—	++	++	—	—	—	+	±	—	—	—
7		1800	male	—	—	—	—	—	++	++	—	—	—	++	±	—	—	—	+++	+++	+	—	—	++	±±	+	—	—	±±	±+	±	—	—
8		2010	female	—	—	—	—	—	++	+	—	—	—	++++	++	+	—	—	+++	++	±	—	—	++	±±	+	—	—	++	+	—	—	—
9		1980	male	—	—	—	—	—	±	+	—	—	—	±	±	±	±	—	++	++	±	—	—	++	+	—	—	—	++	+	—	—	—
10	con-trol	2000	female	—	—	—	—	—	++	++	—	—	—	++	++	±	—	—	+	±	—	—	—	++	±+	±	—	—	—	±+	++	—	—
11		1600	female	—	—	—	—	—	+	+	—	—	—	+	+	—	—	—	++	++	—	—	—	++	±+	±	—	—	++	+	—	—	—
12		2030	male	—	—	—	—	—	++	++	+	±	—	+±	±+	±	—	—	++	++	—	—	—	±+	±+	—	—	—	±+	++	—	—	—
13		1860	male	—	—	—	—	--	+	+	—	—	—	++	++	—	—	—	+++	++	+	—	—	++	++	±	—	—	±±+	±+	+	—	—

Meaning of Symbols: ++++ Complete agglutination, clear liquid with large precipitate; precipitate; + Liquid not clear, very slight precipitate; ± Doubtful reaction; − Negative.

The lack of agreement of the data given above can probably be explained by the fact that prolonged and strong action of a local anesthetic, putting receptors out of action, removes one of the mechanisms of action of antigen on the organism being immunized, as a result of which its immunological reactivity is weakened. But if the action of the local anesthetic is weak, possibly after a temporary inactivation of receptors there is a compensatory strengthening of their function which is the cause of a stronger influence of the antigen on the organism.

The reaction of the body to the introduction of vaccine is not limited to immunological disturbances which have a specific character. In the vaccinated organism phenomena also develop which proceed uniformly and do not depend on the character of the injected antigen. These phenomena, manifesting themselves as temporary distrubances of temperature and inflammatory reactions at the site of injection of the antigen, acquired the designation of general and local vaccine reactions.

The influence of novocaine on the course of the vaccine reaction has been studied even less than its influence on immunogenesis. In the present communication we attempt to fill this gap, and present the results of our observations on the course of vaccine reactions in human beings immunized with polyvalent vaccine containing 1% novocaine. Two ml of the novacine vaccine was injected subcutaneously (Table 1 and 2).

From Table 1 it is evident that a sharp general reaction (temperature rise to 38.6° C or higher) was noted in the control group in 3% of those vaccinated, but in only 1.06% of those vaccinated with novocaine. Reactions of moderate degree (temperature 37.6 – 38.5°) were observed in the second group in 4.86%, but in 11% of the control group. The number of weak general reactions (temperature 37.1 – 37.5°) decreased to 11% as compared to 24.5% in the controls. Novocaine postponed the time of development of general reactions 3 hours on the average.

As is clear from Table 2, local reactions were observed in persons vaccinated with novocaine more rarely than in the controls, and appeared on the average about 12 hours later.

Our observations confirm that anesthesia of the site of injection of vaccine may change the course of the response of the organism, including the vaccine reaction.

After it had been thus established that with the aid of novocaine general and local vaccine reactions are diminished in persons immunized with polyvalent vaccine, we were confronted with the question: what influence does the addition of novocaine to polyvalent vaccine have on the development of immunity in the vaccinated organism. To answer this question we carried out an experiment on animals.

METHODS

For the experiment 13 rabbits were taken. Five animals were immunized with polyvalent vaccine containing 4% novocaine (four times as much as in the vaccine used for immunization of human beings). A fourth rabbit was vaccinated with vaccine containing 1% novocaine. We gave the control group, consisting of 4 rabbits, polyvalent vaccine without novocaine.

The anti-typhoid agglutinins developed in the serum were studied in all the experimental animals. Determinations of these antibodies were carried out before immunization, then about 14, 21, 28, 40 and 60 days after immunization.

Serum titers, for the cases in which anti-typhoid agglutinins were found in the experimental animals, are presented in Table 3, where the degree of agglutination is shown by plus signs (+).

As in evident from Table 3, anti-typhoid agglutinins appeared in the serum of all experimental animals after immunization. The agglutinin titers of the animals vaccinated with novocaine are no lower than those of the controls. It may be concluded that the application in our method of novocaine diminishes the vaccine reactions following immunization with polyvalent vaccine and does not hinder the development of antibodies to any significant extent.

SUMMARY

The influence of anesthesia of the site of introduction of vaccine on the development of vaccine reactions was studied. For this purpose one group of patients was immunized with polyvalent vaccine which contained 1% novocaine. It was established that under the influence of the novocaine local and febrile reactions were greatly reduced. The addition of novocaine had no effect on the immunogenic effect of the vaccine.

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ONOCOLOGY

ON THE SPECIFICITY OF THE SHOPE VIRUS

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That viruses play a definite role in the development of certain animal tumors has been definitely established, but how important they are in the etiology of human tumors is not yet clear.

Tumorigenic viruses are usually considered to possess exceptional tissue specificity. This, however, is completely false. The virus causing cancer of the liver in the leopard frog is known, under certain conditions, to also cause tumors in the tissues of the triton [9]. In this case, tumors develop in the periosteum, joints and liver of the triton. In chickens, lymphomatosis causes great periosteous growths, and when leukemic material is passaged to chickens, endotheliomas, hemangiomas, osteochondromas and sarcomas develop [5].

The "milk factor" has been successfully cultivated on mouse connective tissue [2] and even on human embryonal connective tissue [4]. The skin of rat embryos processed with the Shope virus and transplanted to the brain caused typical papillomatous affections [6].

All these facts suggest that, despite the selective affinity of viruses to this or that tissue, they can adapt to new conditions of existence and multiply in other tissues of the same animal or even in the tissues of a different kind of animal.

Although it is easy to cause cutaneous papillomas in rabbits which become malignant in almost 100% of the cases by means of the Shope virus, to date, no tumors have resulted from an injection of this virus into other tissues or organs. The Shope virus has been injected by different authors intravenously, subcutaneously, intraperitoneally, intracerebrally, into the testicle, the epithelium of the oral cavity, the trachea, the rectum, the urinary tract, the external gonads, and into the mammary and salivary glands [11, 10, 7, 1]. Only with the intravenous injection did papillomas appear on the skin; no tumors were obtained anywhere else.

The negative data of these works permit the proposition that the Shope virus possesses an exceptional tissue specificity, that it is "strictly dermotropic." However, if one considers the data given above from these works, this proposition cannot be considered conclusively proved. It is possible that the Shope virus caused another reaction in the other tissues.

The purpose of this work was to make a histological study of the changes occurring in various tissues injected with the papilloma virus.

EXPERIMENTAL METHODS

Tissue from a Shope papilloma preserved in 50% glycerin was washed free of the glycerin, ground with a glass pestle and diluted with an equal volume of a physiological solution. The resulting suspension was injected into the rabbits (in a dose of 1 ml per rabbit) through a hypodermic needle into certain organs or rubbed into a scarificated mucous membrane.

The submaxillary salivary glands of ten rabbits were injected with the papilloma suspension (the skin was cut open and then sutured); the material was injected into the testicle of five male rabbits, into the bladder wall (after the celiac cavity had been opened) of twelve rabbits, and rubbed into the mucous membrane of the external gonads of 11 animals (7 males and 4 females).

A suspension made from normal rabbit skin prepared in a similar way was injected into the corresponding organs of the control group of rabbits.

The rabbits were observed. The animals were killed and dissected after intervals of from one month to one year; all changes were recorded, and the experimental organs were removed, fixed and histologically processed.

EXPERIMENTAL RESULTS

No tumors were obtained at the place where the papilloma suspension had been injected into the salivary glands of any of the ten rabbits thus injected. Only in one rabbit after 6½ months did two small papillomas appear on the skin in the thoracic region. Their growth was not active, and they disappeared after a short while. The salivary glands of the experimental group grew somewhat larger than those of the control group. Histological examination showed, both in the early and late stages (over 7 months) of the experiments, epithelial growth in the ducts: thickenings and, in places, formations of massive accumulations. No mitoses could be found in them (Fig. 1). No abnormal changes were observed at these same stages in the control experiments.

A papilloma appeared the 4th month on the skin of the right flank in one of the five rabbits injected with the papilloma suspension through the testicle. The papilloma grew actively. No tumors appeared at the place where the material had been injected. Histological examination disclosed no changes in the testicle tissue as compared with the control.

Nor were tumors obtained in the rabbits injected with the papilloma suspension through the thickness of the bladder wall. However, many multinuclear cells appeared in the epithelial layer of the bladder wall, which were different from the ordinary multinuclear cells of a normal transitional epithelium. Normally, they are only found in the sheath layer, but, in the experimental material, they were found in the middle cellular layers as well.

In one field of vision with a small microscopic magnification (objective 8, ocular 7), one could count up to 17 multinuclear cells, whereas only 2-3 are visible normally. The small cells contained 5-8 nuclei, larger cells, about 20, and the giant cells contained up to 40 nuclei. The nuclei were arranged either in a bunch in the center of the cell, or in a chain, if the cell was elongated, and in separate groups in the giant cells. In some multinuclear cells, the nuclei were arranged like a wreath around the periphery. The cytoplasm here was lumpy and coarse, and one could sometimes see vacuoles in the cells (Fig. 2).

No tumors were obtained in the animals rubbed with the papilloma suspension in the mucous membrane of the external gonads at the place where the material was administered. The mucous membrane was histologically normal. Only in one male, a month after the beginning of the experiment, was a node 2 × 8 mm in size discovered where the papilloma suspension had been administered, under the mucous membrane of the preputial sac. This node persisted for 2 weeks, and then disappeared; at that time, an enlarged lymph node in the left groin began to be apparent. By the third month of the experiment, thick, rather inactive nodes could be felt in the celiac cavity.

The rabbit was killed on the 96th day of the experiment. The mucous membrane at the place where the papilloma suspension had been rubbed in was unchanged. An enlarged lymph node, 2.5 cm in diameter was found under the skin of the left groin; two large groups of fused lymph nodes 9 and 4 cm in diameter were found transperitoneally at the base of the mesentery and on the aorta. There were two small, round nodules 2 and 1.6 cm in diameter in the spleen. On cross-sections of the nodes, one could see macroscopically foci of necrosis and small hemorrhages. Live tissue remained mostly along the peripheries of the nodes. In this case, histological examination showed that a tumor had formed.

In all the nodes, the tumor was made up of more or less similar cells, poor in cytoplasm, which were larger than the lymphocytes and distinguished from them by the character of the nuclei. The latter were oval or round and stained rather lightly. Both normal and abnormal mitoses were found in abundance. There was practically no stroma in the tumor.

In other portions of the tumor, along with the small cells with darkly stained nuclei, large cells, which were both uninuclear and binuclear, were found. There were many giant cells containing up to 10 nuclei (Fig. 3).

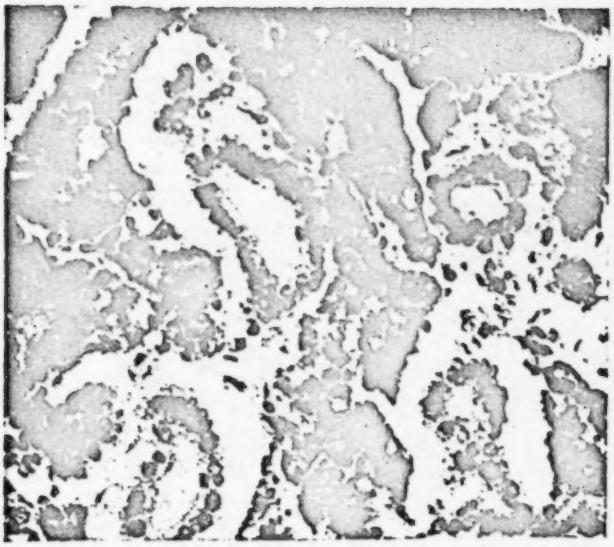

Fig. 1. Section through a salivary gland. 200th day of the experiment. Epithelial growth in ducts of salivary gland. Photomicrograph. Stained with hematoxylin-eosin. Magnified 300 times.

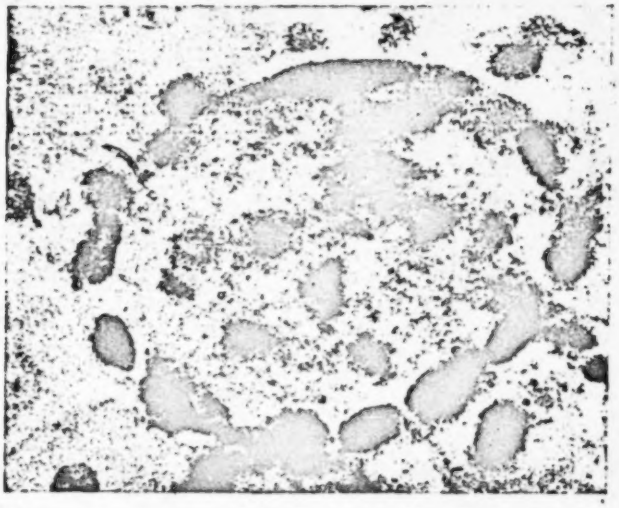

Fig. 2. Total preparation of bladder epithelial sheath. 240th day of the experiment. Giant, multinuclear cell, 14 nuclei of which are arranged around the cell periphery. Photomicrograph. Stained with iron hematoxylin. Magnified 300 times.

Therefore, the tumorous process affected the lymph nodes and was also found in the spleen. The actual tissue of the tumor consisted of rather undifferentiated cells. It was similar to the so-called reticulocytoma.

This tumor was transplanted to 9 rabbits. The results of the transplantations were negative.

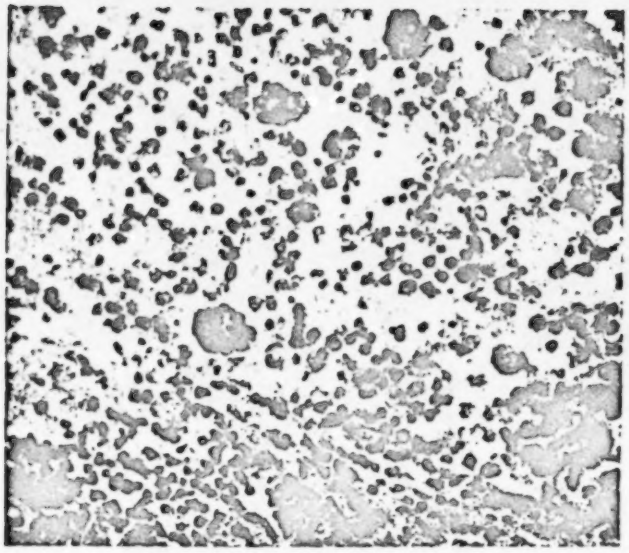

Fig. 3. Tumor section. Large multinuclear cells among small and larger tumorous cells. Photomicrograph. Stained with hematoxylin-eosin. Magnified 170 times.

The data presented deserves attention. The changes observed in the bladder epithelium and in the epithelium of the salivary gland ducts could be considered a peculiar reaction in response to the virus injection. There were no such changes observed in the control experiments.

The Shope virus causes the acceleration and intensification of the growth of rabbit skin explants, disturbs the processes of cell division and causes the appearance of atypical cellular forms [3]. But cell proliferation is not only caused by tumorigenic viruses. It is also observed in other virus infections (chiken pox, neurovaccine, etc.) as one of the stages of the disease [8].

The appearance of giant and multinuclear cells is connected with this "pathologic" proliferation.

The growth of the salivary gland duct epithelium and the appearance of giant , multinuclear cells in the epithelial sheath of the bladder can be considered as a manifestation of "pathologic" proliferation.

The mucous membrane of the external gonads of the rabbit belongs to the cutaneous type of membrane. Its sheath is a multilayer, flat epithelium, which produces mucus. One would expect that here would be the first place at which a papilloma would develop, but such was not the case. A reticulocytoma type of tumor was only observed in one rabbit.

Why did this one develop? In the first place, the tumor could have developed spontaneously, quite apart from our experiments. However, spontaneous tumors are extremely rare in rabbits. For the purposes of this article, one can suggest the following arguments. The tumor originated in the regional lymph node nearest the place of implantation, and then, the process spread to the lymph nodes located along the aorta. Its progress was traced.

Entering the lymph node through the lymph circulation, the virus found a favorable environment in the node. Adapting itself to the new conditions, it began to multiply energetically and was thus able to cause those changes in the tissues which are usually defined as malignant tumorous growths.

However, all these reasons are propositional, since we had only one case to observe. The experiment must be repeated with a similar result in order to conclusively prove that this tumor was caused by the Shope virus.

SUMMARY

Introduction of the Shope virus into different organs of rabbits resulted in histological changes. Profuse epithelial growth was noted in the salivary gland ducts. Many giant, multinuclear cells appeared in the epithelial layer of the urinary bladder. A reticulocytoma type tumor developed in the extraperitoneal nodes of one of the rabbits. The causation of the changes elicited by the Shope virus is discussed.

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HOW SECTIONING THE SCIATIC NERVE AFFECTS THE BLASTOMOGENIC ACTION OF STRONTIUM-89

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The use of radioactive isotopes with powerful cancerogenic action is a valuable method for the reproduction of tumors in order to study their pathogenesis, prophylactics and therapy. The isotopes selectively sorbed into the skeleton(Sr^{89}, Sr^{90}, Y^{91}, Ba^{140}, Ra^{226}), are known to produce primarily bone tumors, which are often localized in the tubular bones of the limbs [1, 3, 5, 6].

The role of physiological factors in the realization of radiation tumors has hardly been studied at all. The question of the role and importance of the nerve connections and their disturbance in the genesis of osteosarcomas has not been studied at all. This fact prompted the authors to take on the task of examining the influence of peripheral innervation on the blastomogenic action of radioactive strontium-89.

EXPERIMENTAL METHODS

Thirty-eight white rats, weighing an average of 145 g, were used in the experiment. A radioactive isotope-strontium-89 – was injected intraperitoneally in a dose of 77 microcuries per rat (0.53 microcuries per g) in a volume of 0.5 ml. Denervation of the right or left hind leg was done in all of the rats on the day of the isotope injection by transecting the sciatic nerve and removing 2-3 cm of it. X-ray pictures were taken twice during the experiments (on the 180th and 262nd days) in order to find any osteosarcoma development as soon as possible. In some of the dead animals, the strontium content in the femur and the tibia of both the denervated and the nondenervated leg was determined.

EXPERIMENTAL RESULTS

The average life duration (LV*50%) of the experimental animals, determined by the method of puncture-analysis (with constituent intervals for R^2=0.05), was about 190 ± 25 days when the rats were injected with 0.53 microcurie per gram of strontium-89.

In the majority of the rats, the skin atrophied and dystrophic ulcers developed in the region of the ankle joint of the denervated leg. Along with the atrophy and the thinning of the bony parts in the region of the metaphysis, a superfluous formation of bone tissue was sometimes observed in the denervated leg. Atrophic processes also developed in the hemopoietic tissue of the bone marrow of the denervated leg. The denervated bone marrow contained almost no hemopoietic cells, and the amount of collagenous fibers in its stroma increased considerably (Fig. 1).

Most of the animals (22 out of 38) unfortunately died before the 200th day, during the so-called "latent" period, necessary to the development of osteosarcoma. Bone tumors, therefore, only developed in a small number of animals. Malignant, osteosclerotic osteosarcomas developed in the leg with the intact somatic innervation of 3 out of 15 rats which died on the 247th, 286th and 316th day (Fig. 2 and 3, A); the tumors developed from the proximal epiphysis of the tibia in two rats and from the diaphysis of the femur in the third.

A bone tumor developed in the denervated leg in only one rat, which died on the 505th day. This tumor

* Transliterated. No explanation given in Russian text.

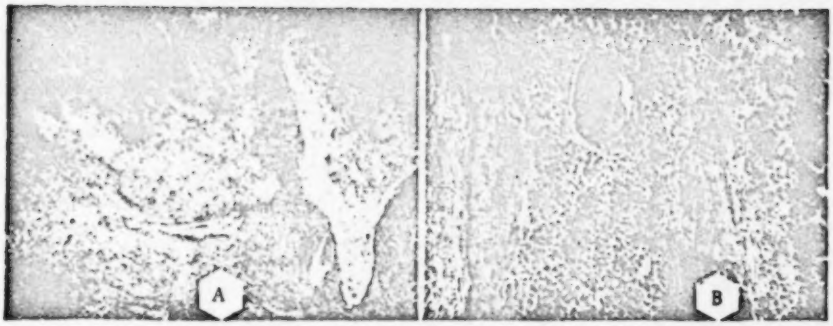

Fig. 1. A) Tissue of the denervated leg of a rat on the 90th day of the experiment with the injection of Sr^{89} (0.53 μ c/g). Aplastic bone marrow. Massive thickening and sclerosis in the region of the metaphysis; B) tissue of the control leg with intact innervation. Hemopoietic bone marrow. Ordinary compact bone.

Fig. 2. X-Ray of rat on 260th day after Sr^{89} injection (0.53 μ c/g). The rat died on the 286th day. Osteosarcoma of the proximal part of the tibia of the nondenervated leg.

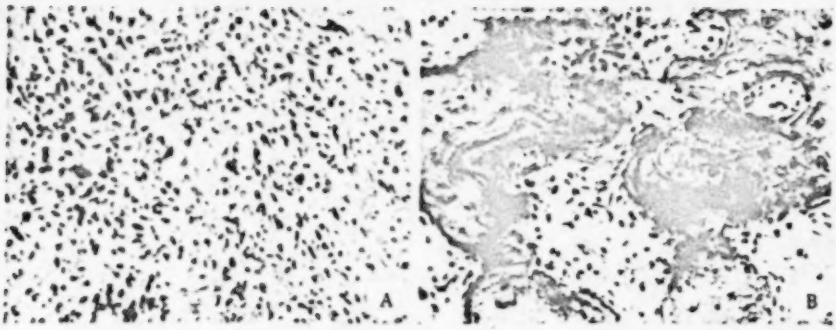

Fig. 3. Condition of tissue of a rat's leg in the experiment with the injection of Sr^{89} (0.53 μc/g). A) nondenervated leg on 316th day of experiment. Osteosclerotic sarcoma; B) denervated leg on 505th day of experiment. Osteosclerotic sarcoma.

not only developed later, but was in a place atypical for osteosarcoma – in the region of the ankle joint, where, for a long time, there had been an ulcerous affection of the skin and subcutaneous cellular layer. This tumor was a benign osteosclerotic sarcoma (Fig. 3,B).

The content and concentration of the strontium-89 was determined in the normal and denervated bones of the hind legs (femur and tibia) in some of the rats.

As the table below shows, the average content and concentration of strontium in the denervated femur was 24% and 14% lower than in the control. Analogous results were obtained with the tibia.

It was interesting to observe that the average weight loss of the denervated femur (10-15%) was more weakly expressed than the decrease in the strontium content (24%). This suggests that sectioning the sciatic nerve causes demineralization of the bone tissue as well as its atrophy.

The strontium content in the femur with the osteosarcoma was 50% higher than in the denervated and control femurs of the rats which died at the same time but did not have tumors, whereas the concentration of Sr-89 was considerably lower (3.5-3.7 times). The relatively lower content of strontium-89 in the denervated femur of rat No. 6) see Table), which was similar to the other denervated bones (according to decomposition per minute), was explained by the higher isotope content in the femur with the tumor.

This previous experiment indicates the important part played by the nervous system in the genesis of the tumorous process. One must mention that sectioning the sciatic nerve did not completely eliminate the ability of the bone tissue to react to the influence of tumorigenic agents, which is indicated by the benign osteosclerotic osteoma found in the region of the ankle joint on the 505th day. We must emphasize that, although we have observed a comparatively large amount of experimental material, including more than a thousand cases of tumor, this was the first time that we have observed the action of a radioactive isotope to cause a benign osteoma.

Let us turn to the unusual location of this tumor. As we have already observed, osteosarcomas ordinarily develop from the epiphysial region of the tubular bones. Evidently, a long unhealed, dystrophic ulcer of the skin and underlying tissues in the region of the ankle joint was the factor which, when combined with the action of the strontium-89 stimulus, gave rise to the benign osteoma.

Denervation appears to limit the blastomogenic reaction of bone tissue to the action of the specific stimulus of strontium-89. This conclusion is confirmed by the results of experiments recently conducted with cerium-144. These experiments showed that transecting and removing part of the sciatic nerve immediately after an injection of cerium-144 lowers the rate of osteosarcoma development in the denervated extremity by two times. The results obtained are known to agree with the observations of experimental and clinical oncology, which has long used the method of sectioning and removing nerves. For example, A. G. Molotkov [2], by sectioning the second branch of the trigeminal nerve, obtained, in some cases, rapid healing in skin cancer of the cheek and upper lip in humans. According to the observations of I. A. Pigalev [3], sectioning the vagus nerves hinders, but does not eliminate the development of gastric melanoadenomas.

Relative Content (in entire femur) and Concentration (per gram of wet weight) of Strontium in the Denervated Femur (in % of control)

No. exptl. animal	Time of rat's death	Content	Concentration
1	193	62 5	77 5
2	196	82 4	89 0
3	223	79 3	102 0
4	225	77 0	86 0
5	236	70 4	80 5
6	247*	58 1	344 0
7	273	67 4	69 6
8	291	79 6	93 2
9	311	81 7	83 5
10	338	84 2	92 2
Average amt. without rat No. 6.		76±2 5%	86 0±3 2%

* In the femur of the control leg of rat No. 6, an osteosarcoma was found; the weight of this femur was 6 times greater than that of the denervated one.

What is the explanation for the inhibition of osteosarcoma development in the denervated leg ? It is certainly not due to any effect of denervation on the distribution of the radiator, since there was very little difference in the isotope content of the normal and denervated bones, and, moreover, osteosarcomas can develop from smaller doses of strontium as well. The comparatively rare development of osteosarcomas in the denervated leg is evidently connected with the general inhibition of growth and the preeminently atrophic changes of the bone tissue caused by denervation (atrophy and thinning of bony parts, lowered mineral salt content), which changes naturally hinder processes causing swift and uncontrollable cell growth, such as the tumorous process. Therefore, in the final analysis, the "favorable" effect of denervation is explained by its inhibiting influence on bone tissue growth and development.

SUMMARY

The role of sciatic nerve section on the blastomogenic effect of strontium-89 has been studied in albino mice. Three out of fifteen animals developed malignant tumors on the limbs with an intact somatic innervation. But only one animal developed a tumor on the denervated limb, on the place of an unhealing trophic ulcer. The tumor was a benign one, an osteosclerotic osteoma. The results could not be accounted for by any difference in the strontium content of the normal and denervated limbs since the difference was comparatively small.

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EXPERIMENTAL BIOLOGY

THE REGENERATION OF LYMPH NODES AFTER THEIR PARTIAL REMOVAL OR INJURY

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The regeneration of lymph nodes and the possibility of their re-formation is of real theoretical and practical interest. This is a question which has been much discussed but which is still far from being solved, and the opinions of different researchers are extremely contradictory. A. P. Avtsyn [1], J. Auche [2], E. Clark and E. Clark [6], S. Groot [8], J. Horii and T. Sakai [10], M. Ohkubo [14] and others believe that lymph nodes are capable of regeneration. H. Baum [4], P. Heuter [9], A. W. Meyer [11], L. Moriconi [12], G. Ottaviani and M. Cavalli [15], A. G. Sanders and H. W. Flory [20], A. Vecci [22] and others disagree with this.

Data describing the conditions favorable and unfavorable to lymph node regeneration are extremely limited [7, 17, 19].

The opinion that lymph nodes completely lack the ability to regenerate is contradictory to all the accumulated clinical and experimental data, which indicate that these organs are highly reactive. It is difficult to believe that lymph nodes, which change sharply in pathologic conditions and are made up of plastic lymphoid tissue, are unable to either regenerate or form anew.

EXPERIMENTAL METHODS

In this article, we present data on the regeneration of injured iliac lymph nodes in white rats weighing 100-130 g, depending on the degree to which lymph circulation to these nodes was disturbed (experimental series I, II, III and V) and on the degree to which the nodal tissue was altered (experimental series IV). In experimental series I, the lymph nodes were seared in one or several places (2-5); in series II, from 1/5 to 1/2 of the lymph node was resected. In all of the experiments of this series, the part of the lymph node with the afferent lymphatics was left on the right side, and on the left side, the part with the efferent lymphatic was left; the afferent lymphatics, for a distance of 2-3 cm of their lengths and the corresponding portion of the node were removed. In experimental series III, the middle portion of the nodes was left, and their poles with the afferent and efferent lymphatics removed. The lymphatics were dissected out and cut at a distance of 2-3 cm. In experimental series IV, after half of the node had been removed, the remaining part was seared along the line of the cut. In series V, 1/3-1/4 of the lymph node was left, and, at the same time, the iliac or the femoral vein was carefully separated to avoid damage to the near-by lymph vessels and ligated below the node. The amount of lymph circulating from the limb into the node was greatly increased in these experiments due to the stoppage of circulation through the vein. Fifty experiments were conducted in all (9 experiments in series I, 17 experiments in series II, 9 experiments in series III, 10 experiments in series IV and 6 experiments in series V). Most of the rats were killed after intervals of up to 30 days after the operation, a few, after 2-3 months. Histological processing was combined with Trypan blue or India ink injections of the lymph system. The first injection was done during the operation in order to render the lymph bed more completely visible; a second was sometimes done with the second laparotomy to show the changes occurring after the operation, and third injection was done when the animal was killed. The lymph nodes were measured before and after the operation in all the experiments.

EXPERIMENTAL RESULTS

All the experiments in series I had similar results: the lymph nodes regenerated in the seared places as early as 10-12 days after the operation and could not be distinguished from the undamaged nodes. No deformation of the nodes due to the searing was observed. When the nodes were injected, the color spread evenly; there were no portions to which the color did not penetrate. Along with regenerated lymph tissue in the formerly damaged nodes, microscopic examination frequently showed connective tissue rods growing up from below into the body of the node. These rods, however, never became very thick; subsequently, they became somewhat thinner, looking like thin bands along the greater part of their length, sometimes somewhat thickened near the capsule. In other cases, the connective tissue rods were only preserved on the periphery of the nodes and did not penetrate very deeply. Without going into a detailed description of the course of regeneration in the damaged part of the lymph nodes, we shall only mention that the reticular syncytium proliferated first of all, growing further and further beyond the undamaged places as the wound healed. This syncytium, distinguished by the extreme polymorphism of its component elements, contained a varying amount of lymphocytes, distributed at random, most of which probably infiltrated from the remains of the node. At the same time, foci of lymphopoiesis, containing lymphocytes in all stages of formation and differentiation, appeared in the syncytium. At first, these foci were distributed apart from each other, later, however, as they grew and changed form, they grew together. Sinuses were gradually differentiated, and their connection with the sinuses of the undamaged part of the lymph node was established. The color injections confirmed the latter. In spite of known variations in the different experiments, the general course of regeneration was homologous in all of the experiments.

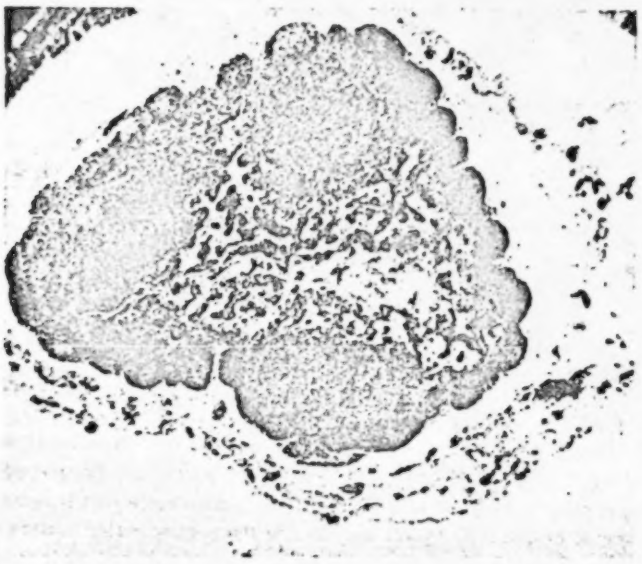

Fig. 1. General view of iliac regional lymph node of a white rat which was completely regenerated by the 23rd day of the experiment after 3/4 of it and the efferent vessel had been resected. Photomicrograph. Magnification - 15 times.

In experimental series II, the remaining portion of the lymph node with the afferent lymphatics (on the right) had, after 15-20 days, become a macroscopically well-formulated node, easily filled with color by the injection (Fig. 1). The color extended from the node along the efferent lymphatic, which was usually located at the pole opposite to the afferent lymphatics. This vessel was evidently formed from the regeneration of the remaining part of the efferent lymphatic, which grew up to meet the remnant of the node. Sometimes, one of the afferent lymphatics became the efferent.

The course of regeneration was generally analogous to that described earlier: first the reticular syncytium

began to grow, and individual foci of lymphopoiesis appeared. The largest of these appeared along the periphery, near the differentiated capsule. Then they increased in size. The individual foci in the center of the node also became larger and grew together. At the same time, the number of blood vessels which had appeared at first among the elements of the reticular syncytium decreased somewhat. The remnant of the lymph node also underwent a known reconstruction. In it, during the first few days after the operation, the normal relationships between the sinuses, myelinated septa and follicles were disturbed; the contours of the latter became more defined. A considerable amount of lymphocytes passed into the intermediate sinuses, thus concealing their contours. The preserved part of the lymph node became more and more like an unformed accumulation of lymphoid tissue. The marginal sinuses, on the other hand, became much larger. However, this process gradually ceased, and by the 20-25th day after the operation, at the latest, (often before the 15th day), the normal structural picture was regained. Regeneration always occurred, although, in some cases, it was very slow. The size of the regenerated node varied in different experiments; in some cases, it was only 2/3 as large as the original node, and in others, it was either about the same size as before or only slightly smaller. Only in one experiment did the regenerated node remain very small. Histological examination of this node on the 20th day after the operation showed a structure typical of this stage, but the regenerated part was still not clearly delimited from the surrounding connective tissue, and there was no capsule at this place.

Where the portion of the lymph node including the afferent vessels and the afferent lymphatics themselves had been resected for a length of 2-3 cm (on the left), results analogous to those already described were obtained in 2 cases out of 11. The injection showed that, in these two experiments, the lymphatics from the opposite side, where the part of the node with afferent lymphatics remained, grew onto the remaining portion of the node. There was no regeneration in the other 9 experiments. Connective tissue grew along the line of the cut; the lymph node remnant became disorganized, causing it to look at first like a non-differentiated accumulation of lymphoid tissue, lacking sinuses and without the follicles with their centers of division (Fig. 2). There were different numbers of macrophages in different portions. Lymph circulation through the node was not restored, since the regenerated lymphatics were located some distance from it; more and more connective tissue grew from the remaining portion of the node; the number of lymphocytes decreased sharply, and the lymphocytes themselves were distributed in the form of separate islands, differing greatly in shape and without definite borders, due to the gradual migration of lymphocytes beyond the limits of the lymph node remnant.

The 9 experiments in series III gave the following results. The several injections of Trypan blue showed that all of the lymphatics approaching the node could not be distinguished in 3 experiments. The lymph circulation seemed rather disturbed, and the lymph nodes regenerated, but quite slowly. Two of the nodes remained small, and four other approximated those observed in experimental series II on the right in size. Regeneration did not occur in the other 6 experiments, and the results in these experiments were the same as those observed in the 9 experiments of series II in which the operation was done on the left.

The results obtained in experimental series IV were generally the same as those obtained in the experimental series II with the operation on the right, i.e., without the searing. In these experiments, regeneration sometimes occurred somewhat later, and the sizes of the regenerated parts were smaller. In experimental series V, on the other hand, regeneration was accelerated, and signs of a compensatory reaction in response to the intensified lymph circulation were often found in the regenerated lymph nodes, which signs we have already described (sinus expansion, proliferation of reticular syncytium, etc. ([A. I. Braude, 1956]).

After considering all the data presented, one can conclude that the remaining part of a lymph node can only regenerate when lymph circulation through the node is continued (experimental series I) or when it is comparatively quickly restored (experimental series II with operation to the right). On the other hand, regeneration does not occur when the lymph circulation is cut off, and the remaining part becomes gradually reduced and is replaced by connective tissue. This occurred in experimental series III and the experiments in series II with the operation on the left, when the collateral lymph circulation which developed by-passed the remaining part of the lymph node. On the other hand, when the node's connection with the lymphatics was restored (by the growth of the lymphatics from the opposite side, or due to their incomplete resection), in two experiments of series II with the operation to the left and in three experiments of series III, the node regenerated. Regeneration occurred especially rapidly when lymph circulation through the node was intensified (experimental series V).

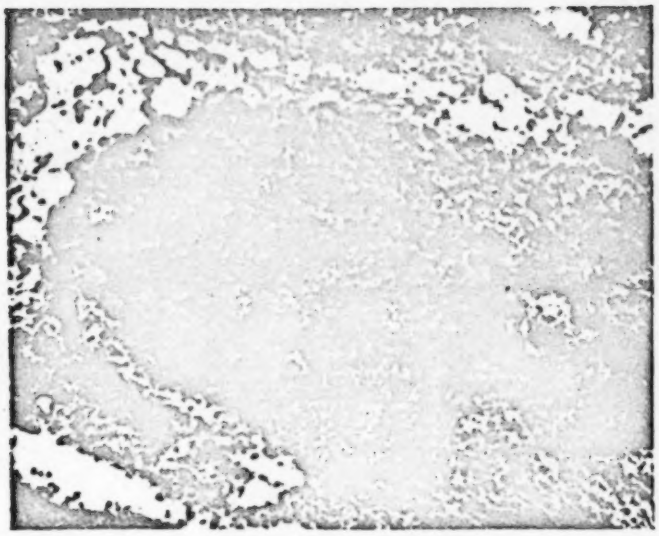

Fig. 2. Disorganization of iliac lymph node remnant in white rat, under conditions of lymph circulation stoppage, on the 20th day of the experiment. Photomicrograph.
Magnification – 120 times.

The degree to which the nodal tissue was altered affected the experimental results. The data received in experimental series IV and II with the operation to the right were generally analogous, except for the fact that the sizes of the regenerated nodes varied somewhat. However, this circumstance alone is not sufficient basis for any sort of conclusion. In the course of the experiments in series II, III, IV and V, additional lymph nodes usually appeared at a greater or lesser distance from the regenerated nodes. Due to their appearance, the lymph flowing out from the hind leg entered several (as many as 3-4) iliac nodes on each side instead of just one, as happens normally. Therefore, there was somewhat less lymph circulating through the regenerating node than before the operation. It seems likely that this phenomenon, which was expressed to varying degrees in the different experiments, was the reason that the regenerating node was not always as large as the original node. Turning back to the results of experimental series IV, it should be emphasized that the degree of injury to the lymph nodes evidently has less influence on their regeneration than the preservation or disturbance of lymph circulation. The latter has the deciding role in the process studied. With this in mind, we consider it impossible to consider the question of whether or not lymph nodes are capable of regeneration after being injured or partially removed without considering the concrete conditions in which regeneration occurs. Our study of regeneration clearly demonstrated the constructive role of lymph circulation through the node, in the absence of which, the node not only does not regenerate, but changes from a structurally complicated organ into an accumulation of lymphoid tissue, lacking a clear contour and containing a greater or lesser amount of macrophages. As opposed to the comparatively brief disorganization stage occurring in the lymph node remnant during lymph circulation disturbance, these phenomena appear to be irreversible.

SUMMARY

A series of experiments were conducted on albino rats, weighing 100-130 g It was established that iliac lymphatic nodes are able to regenerate after injury or removal of their greater part. However, regeneration takes place only in conditions of continued circulation of lymph in the node or in relatively rapid recovery of the circulation. In stable obstruction of lymph drainage, on the contrary, there is no regeneration in the lymph node and gradual fibrosis takes place in its residual part.

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METHODS

CINEMATOGRAPHIC RECORDING OF VISUAL REFLEXES AND ITS DIAGNOSTIC SIGNIFICANCE

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Visual reflex disorders are one of the main symptoms in neurosyphilis and certain other nervous diseases. But the absence of a light reflex observed in the Argyll Robertson syndrome is the final stage. A number of intermediate stages are frequently encountered in clinical practice, from those which are slight and scarcely perceptible by eye up to complete absence of reflexes (eyes immobilized). The subjective evaluation of visual reactions, even when various pupilloscopes are used, cannot give exact data on the nature of the disorders.

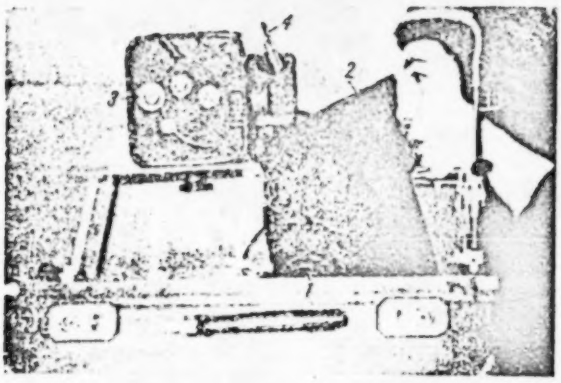

Fig. 1. General view of the apparatus (explanation in text).

Bellyarminov's principle [2], used in Glezer's apparatus [3] and by Zelondzhev [4] and the photoelectric methods of Mattes [9], Cuppers [6] and Ataev [1] require extremely rigorous conditions, the head and eye of the subject having to be immobilized.

Loewenfeld's special check experiments [7] showed the photoelectric method to be inaccurate.

We used a cinematographic method of serial recording for the visual reflexes in neurosyphilis. An advantage of cinematography is that it is reliable. It also, unlike other methods, does not require the head and eye to be strictly stationary. This is very important for studying visual reflexes under clinical conditions.

But several difficulties occur in using cinematography: high illumination is required; the apparatus is cumbrous; the noise and the apparatus constitute additional disturbances; transcribing the curves from the frames is laborious and time-consuming [8].

These shortcomings were eliminated in our method. A portable apparatus was constructed. Here a weakly illuminated background (25-lux at eye level) is lit by two light sources (Figure 1,1) separated by a special baffle (Figure 1,2) so the light from a source fell only on the eye on the same side. The direct and conjoint reflexes could then be studied.

To apply a light stimulus either source could be brightened by a factor 10, to give 250 lux, the frames being taken at 10 per sec. The gaze of the subject was fixed on a small lamp reflected in a mirror (Figure 1,4), this preventing convergence reactions. The noise of the apparatus (Figure 1,3) was eliminated by fitting a sound-absorbing shield.

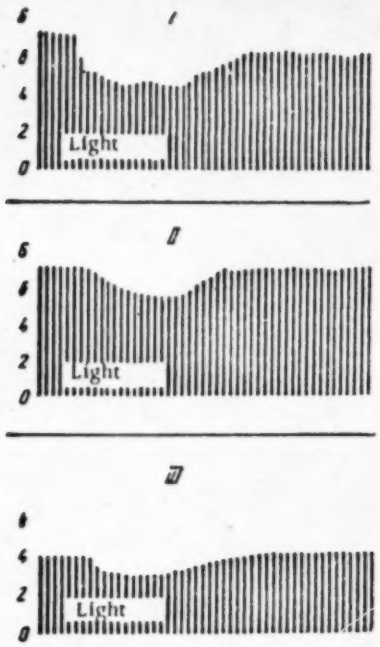

Fig. 2. Pupil reflexes to light.
The traces marked "light" indicate when the light stimulus is operative. The left hand scale is the absolute pupil diameter in mm.
I) Light reflex in normals (latent period 0.2 sec). II) Light reflex in a patient with neurosyphilis (latent syphilitic meningitis). Increase in latent period of light reflex (to 0.5 sec). III) Light reflex in a neurosyphilis patient (syphilis of the brain). There is myosis, increase in light reflex latent period (to 0.4 sec) and reduced reaction amplitude.

The pupil diameters can be measured on the frames and plotted on a graph. An apparatus to do this automatically has been built [5] to record photographically the visual reactions directly from the frames. In this the pupil image is set to have the same diameter as the outlet slit. The pupil diameter is printed on photographic paper or film via this slit, the photographic material running in a film camera. The sequence of readings gives the pupil diameter as a function of time. The curve (Figure 2,1) so obtained consists of vertical lines representing the pupil diameter. The marks are at $1/_{10}$ second intervals (at 10 frames per second in the original camera).

To test this method we studied the light and convergence reflexes in 40 apparently normal individuals and in 30 patients with neurosyphilis.

With this method pupil reactions and eyeball motion in convergence can be recorded simultaneously. It was found that the pupils do not contract instantly in convergence, there being a definite latent period, contrary to the opinion so far current (Figure 3).

Using cinematography minor disturbances in the visual reflexes not detected by eye were found in pathological states. The duration of the latent period cannot be determined visually, or the rate at which the pupils react to light. Objective recording shows that the pupil reflex to light has a latent period lasting from 0.2 to 0.3 sec. in normals (Figure 2,1).

In neurosyphilis patients the latent period was found to be raised to 0.4-0.6 sec. (Figure 2,II), which shows the diagnostic value of this index. The amplitude of the pupil reaction varies from 1 to 2.5 mm in normals. The patients we studied gave various degrees of amplitude reduction (Figure 2, III) as far as complete absence of the reflex. The light reaction time varied from 0.5 to 0.8 sec. in normals.

By dividing the amplitude of pupil contraction by the reaction time we get the rate of the light reaction.

Using cinematography and weak background illumination we could not only record pupil contraction on light stimulation for one eye but also how the pupils enlarged after closing one eye. We could also determine the pupil state in complete darkness. To do this the camera began to run before the lights were switched on. The dark diameter of the pupils could be judged from the first frame exposed. The dark diameter varied from 6.7 to 9 mm after 3 minutes in darkness with normals.

Since cinematography makes it possible to record direct and conjoint pupil reactions to light the relation between these reactions can give one information on the location of damage to the reflex arc. Finally, our method makes it possible to detect minor disturbances not seen by eye, and it may be of diagnostic value in various nervous diseases.

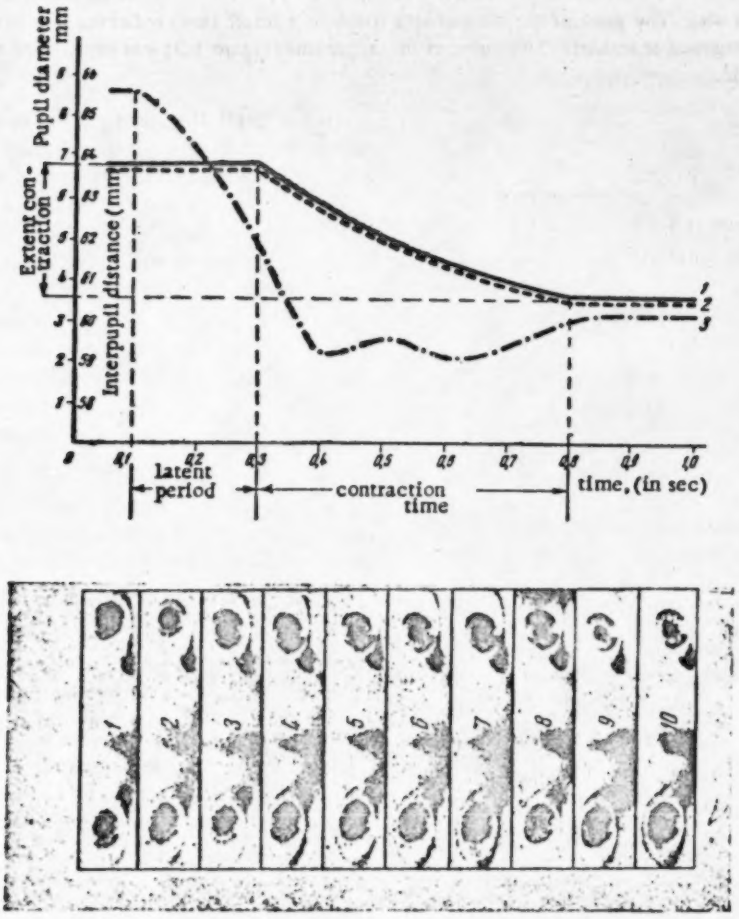

Fig. 3. Reaction of pupil to convergence.
1) right eye reaction; 2) left eye reaction; 3) convergence.
The ordinates are the pupil diameters and interpupil distances, which represent the degree of eyeball and visual axis rotation in convergence. The abscissae are times in seconds; the bottom plate shows the frames from which the graphs were drawn.

SUMMARY

An objective method was developed of cinematographic recording of pupil reflexes in weak light. An apparatus has been constructed for objective photographic recording of pupil reaction curves from motion picture frames. The changes of pupil reactions to light and convergence were investigated in normal conditions as well as in patients with neurosyphilis.

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ISOLATED POUCH OF THE ANTERIOR GASTRIC WALL

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The isolated Pavlov gastric pouch undoubtedly presents the basic preparation available for the study of the secretory function of the stomach. Therefore it is no wonder that the perfection of the technique of its preparation has occupied the attention of many physiologists. At the present time there are over 20 material modifications of the original operation performed by I. P. Pavlov.

Many of these maneuvers are successful in supplementing each other.

However, with all the seeming diversity of the described modifications, each of them directs itself specifically to a search for a better solution of one or another of the following problems.

1. Simplification and perfection of operational technique.

2. Reinforcement of the barrier separating the pouch from the stomach itself.

3. Assuring the maximum preservation of the pouch innervation.

4. Guarding against or, at least, minimizing the digestive effects of the gastric juices upon the skin around the pouch orifice.

5. Forming the pouch in various portions of the stomach.

M. P. Brestkin and V. V. Savich [1] have shown that a pouch fully separated from the gastric body (Fig. 1, II, A and B) preserves enough branches from the vagus nerve as they pass over the greater omentum. With such a disposition, there is no need for forming such connections as musculo-serosal bridges. The technique of this procedure is simple and differs but little from the Heidenhain operation.

A. I. Manuilov [3] proposed a new method for reinforcing the bridges made during the classical operation. He attains this by means of a few simple submucosal isthmic sutures, which, when tightened, form a continuous muscular cylinder between the vaults of the main stomach and the pouch.

V. N. Boldyrev (for description see article by Ya. A. Egolinsky) introduced interesting modifications into the technique of pouch making. To isolate the pouch, he incised all the layers of the anterior wall along line ab; through this opening he cut the mucosa of the posterior wall along line ba, as shown by dotted line in Fig. 1, III. The upper edge of the incision made in the mucosa of the posterior wall is sutured to the upper edge of the incision made in the anterior wall, while the lower edge of the mucosa of the posterior wall is correspondingly fastened to the lower edge of the incision that has been made in the anterior wall. As a result of this, the openings of both the stomach and the pouch become approximated, the innervation of the latter having been assured (as far as the fibers traversing the posterior wall of the stomach are concerned). Unfortunately, this method destroys that portion of the pouch innervation which connects with the pouch formed from the anterior wall.

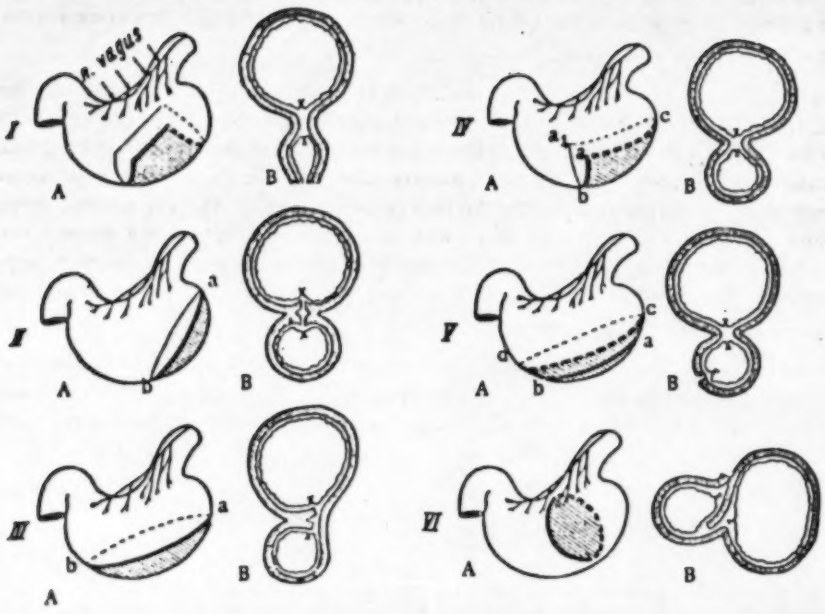

Fig. 1. Basic modifications of the Pavlov pouch. Schemes: A) continuous heavy line – incision through all the layers of the anterior wall; continuous fine line – incision through all the layers of the posterior wall; heavy dotted line – incision through the mucosa (without damage to the muscle and serosal coverings) of the anterior wall; fine dotted line – incision through mucosa of the posterior wall. Schemes: B) diagramatic transections of the corresponding modifications. I) Operation of I. P. Pavlov, A. I. Manuilov, N. N. Samarin and many others; II) Savich-Brestkin operation; III) V. N. Boldyrev operation; IV) Hollander and Jemerin operation; V) Gregory, Hallenbeck and Code operation; VI) operation described in this article.

In the thirties, numerous modifications were proposed [7, 8, 17], the N. N. Samarin [7] method deserving particular attention. It has technical virtues and an original method for securing the barrier between the stomach and the pouch by means of an omental transplant. The B. M. Sokolov operation basically surpasses the methods proposed many years later by R. A. Gregory, G. I. Hallenbeck and C. F. Code [16], as it preserves an excellent innervation of the pouch. It seems that the attendant technical difficulties have prevented more widespread use of this operation. K. M. Bykov and G. M. Davidov [2] were the first to isolate the lesser curvature of the stomach. The technique of isolating the greater and lesser curvatures of the stomach was perfected by A. V. Solovyev [9, 10]. These studies have permitted clarification of the interrelationships existing in the secretory activities of different stomach regions.

It may be stated that after the above-mentioned studies and techniques introduced by N. P. Pyatnitsky [5], Yu. N. Uspensky [12] and V. E.Robinson [6], the techniques for preparing Pavlov pouches attained a high degree of perfection.

In 1938, there were published new modifications of the Pavlov pouch which took into account the anatomical studies made by Hollander and E. E. Jemerin [18]. These investigators proved that the dorsal and ventral branches of the branch of the vagus nerve in dogs is disposed symmetrically along the lesser curvature, as is indicated in Fig. 1,A. From these basic stems arise secondary branches which lie perpendicular to the long axis of the stomach. This circumstance has great significance, as at the time that I. P. Pavlov was developing his operation it was thought that only the ventral branch of the vagus coursed along the lesser curvature and that the dorsal branch curved around the stomach following the greater curvature. It now appears that the innervation of the classical pouch is mediated not through a basic dorsal branch, as thought formerly, but by means of some secondary branchings. In order to preserve more of the pouch innervation, F. Hollander and E. E. Jemerin [18] proposed

their modification (Fig. I, IV, A, B). The incision through all the anterior and posterior wall layers is made from a to a_1, in a direction parallel to the course of the secondary branchings. This preserves the basic mass of the parasympathetic fibers.

Later, this operation was modified by O. Cope, C. E. MacMahon, A. Hogström, R. H. Thompson [15], Neuwelt, Olson, Necheles (1940) [20], as well as others: Gregory, Hallenbeck and Code (1942) [16]. This latter modification is distinguished by its relative simplicity and the excellent preservation of the pouch innervation. The lumen of the stomach is opened widely by an incision along the line ab, and then the mucosa is incised along the anterior and posterior walls following the line cd (see Fig. 1, V, A). The mucosal edges made in the anterior and posterior walls are sewn to each other, thus isolating the pouch from the stomach itself. The integrity of the pouch is restored by a double row of sutures placed in the first incision. In order to prevent hemorrhages, soft compresses are placed (before beginning the operation) upon the greater omentum, and along the line paralleling the expected incision in the mucosa.

As a rule, in these later modifications the muscular bridge is formed by the entire gastric wall. This is quite irrational, as it furnishes no advantages when the lesser curvature is isolated. As can be seen from Fig. 1, V, B, the barrier between the pouch and the stomach itself is least dependable in the last two instances. Their great advantage lies in their excellent innervation.

It is not difficult to observe that, in the majority of instances, either the entire greater curvature or a portion of it is isolated [1, 3, 5, 6, 7, 9, 12, 14].

The majority of the authors employ the classical incision.

It appears to have been firmly established that the innervation is best preserved when the pouch is taken from the most distal portion of the stomach possible.

In all the operative variations it is feasible to apply metallic fistulae to the pouch. This protects the anterior abdominal wall from the corrosive action of the gastric juice and thus avoids the need of introducing drains into the pouch lumen.

After the studies made by K. M. Bykov, G. M. Davidov and A. V. Solovyev, it is difficult to believe that the isolated pouch taken from the greater curvature reflects accurately the secretory activities of all the different portions of the stomach. At the present time, as a rule, the research worker does not see either the region of the lesser curvature or the broad region lying between the greater and lesser curvatures.

It might be thought that it would be more rational to so modify the classical operation that the pouch would embrace both the greater and lesser curvatures with the intermediate regions and could also be made in different portions of the stomach. This is the goal of the operation about to be described. It should be remembered that attention has to be paid to preserving a reliable isolation with an innervation as intact as possible.

Differing from previously advanced operations, we propose to make our pouches from various regions of the anterior wall only and do not demand a direct association with the greater and lesser omenti. Essentially, we form an artificial fold in the anterior wall of the stomach. Figure 2 depicts the beginning and completion of the operation while Fig. 3 shows diagrammatically all the stages of the operation.

After the usual midline incision, the stomach is lifted from the wound. The portion destined to form the pouch is drawn ventrally, the left hand being used, and the fold which forms as a result of this maneuver is clamped at its base with a rubber covered soft clamp. Figure 2, A shows the external view of such a portion of the stomach while Fig. 3, 1 gives a transverse view. Special attention must be paid to having the upper pole of the pouch be slanted towards the pylorus while its lower pole points towards the body of the stomach, as in such a situation there is the least damage done to the innervation of the isolated pouch. At a distance of 4-5 mm above and parallel to the clamp, an incision is made through all the stomach layers directed to the pylorus and greater curvature (Fig. 2, A). The other wall remains untouched and serves later as the source of the nutrition and innervation of the isolated pouch (Fig. 1, VI, A). The next step is to infiltrate, from the mucosal side, the submucosa of the intact wall of the pouch in such a manner that, parallel to the clamp, there is raised a ridge having a thickness of 1.5-2 cm. The exact technique has been detailed elsewhere [12]. As a result of infiltrating the submucosa with novocaine or physiological saline, the mucosal rugae unfold, and there is formed between this and the underlying muscle layer a thick stratum of indifferent tissue (Fig. 3, 2). The scalpel is used to make incisions

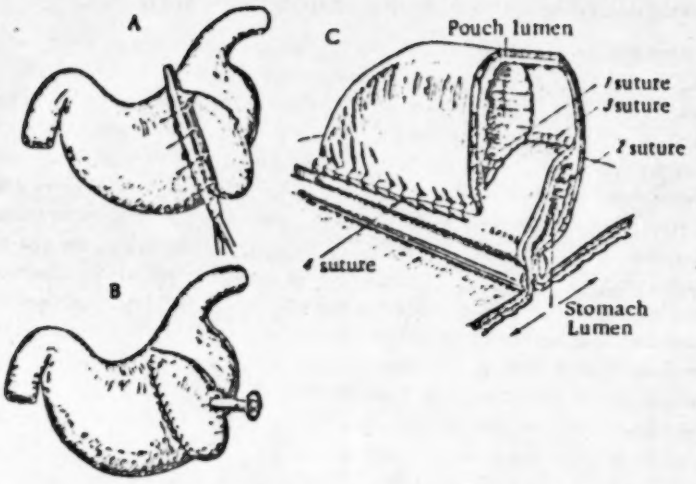

Fig. 2. Beginning and completion moments of the operation. A) Stomach with the fold of the anterior wall compressed by the clamps; arrows point to incision through all the layers of the wall, facing the greater curvature and pylorus; B) external view of the isolated pouch at the end of the operation; C) partially schematic view in transection. Shown are the interrelationships of the different layers of the stomach and the pouch and the location of the four rows of sutures relative to each other.

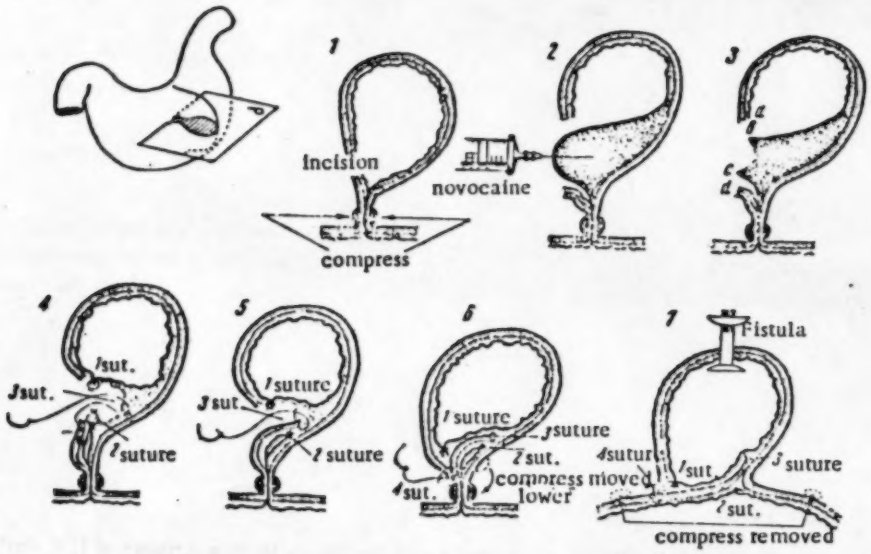

Fig. 3. Basic steps of the operation shown in diagrammatic transections. 1) Section through all the layers of one wall; 2) infiltrating the submucosa of the other wall; 3) a portion of the mucosa is removed; 4) the lumen of the pouch is closed (1st stitch) as is the lumen of the stomach (2nd stitch) and the 3rd (isolating) stitch is applied; 5) formation of an additional muscle barrier between the pouch and stomach as the 3rd row of stitches is tightened; 6) hermetic sealing of the serosal-serosa suture line; 7) transverse view after removal of the clamps and formation of the pouch fistula. In the left upper corner are shown the projections (P) from which the planes of the diagrams are taken.

(both parallel to the clamp and separated from each other by 3-4 mm) which cut through the mucosa and partially also through the infiltrated submucosa. This is shown diagrammatically in Fig. 3, 3.

In this fashion, there are formed four free mucosal edges from which two (a and b) will, in the future, form the dome of the pouch, while two others (c and d) will form the vault of the stomach. The usual scanty mucosa-to-mucosa suture unites edges c and d. Such a suture line cannot pretend to have a hermetic seal. The edges of the mucosa forming the pouch (a and b) are joined with a scanty mucosa-to-mucosa suture; this forming the lumen of the pouch and the first suture line (Fig. 3, 4). A complete hermetic seal is assured by two continuous sutures: serous–submucosal and submucosal and serosal–serous. The first of these gives dependable isolation of the pouch from the stomach, the sutures being taken in the infiltrated submucous stratum and the cut edge of the serous and muscular coats of the stomach (Fig. 3, 4; 3rd stitch). When this suture row is tightened, the mucosa of the stomach becomes buried deeply, the innervation of the pouch remaining unaffected. The second of the two, the last serosal–serous suture line, is placed at the edge of the cut layers of the muscle and serosa of the pouch and the imaginary line traversing the serosa muscle coverings of the stomach, parallel to the preceding stitch and 3-4 cm away from it (Fig. 3, 4th suture row). Usually, after application of the serosal–submucous row of stitches, the clamp is moved some 2-3 cm further from the pouch (Fig. 3, 6). As a result of the application of the last two rows of sutures, the mucosa stitch lines in both the pouch and stomach move and separate from each other, while between them are insinuated the serosal and muscle coverings coming from the stomach side (Fig. 2, B; 3, 6; 3, 7). As a result , there is formed a very adequate barrier composed of two mucosal and one serosal–muscular layer.*

In instances when a large gastric area requires isolation, two clamps may have to be applied. The one or both clamps remain closed until the 4th suture row has been placed, this assuring a clean operative field and hemostasis. This makes it unnecessary to place preliminary vessel sutures. In this operation, the vessels most frequently injured are those coming from both curvatures to the edges of the first incision and they are sealed when the 4th (serosal–serous) row of sutures is tightened. When the clamps are removed, hemostasis is usually excellent. Only in occasional instances is it necessary to apply an extra stitch or two.

Through the opening into the pouch a fistula tube is inserted to the center of the pouch; the inner lumen of the tube has a diameter of 5-8 mm. In most cases the ordinary intestinal fistula cannula sufficed. After insertion of the cannula, the area of the serosal–serous row of stitches is peritonealized by being covered with the greater omentum.

The operation is relatively simple and can be performed in 25-35 minutes. The postoperative course must follow the diets accepted for use after gastric surgery.

The pouch functions well and clearly shows that it has intact innervation. The latent period of secretion in response to meat and bread is 3-7 minutes. When stimulated with meat, the conditioned-reflex response in the pouch produces a secretion of acid and gastric juices. The secretion curve differs from the classical response by being somewhat greater in the first 2-3 hours.

SUMMARY

Another modification of the original Pavlov pouch is presented. The exact details are given. The operation is described as being simple, and the innervation seems to be maintained intact.

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* Profound thanks and full credit must be given to the artist, V. N. Lebedev, who drew the figures accompaning the present study.

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AN IN VIVO METHOD OF OBSERVING NERVES IN FROG URINARY BLADDER

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Vital microscopy enhances the opportunities offered the histologic experimenter and aids in the correlation of morphological and physiological knowledge concerning the animal and human organism.

Real progress in achieving in vivo studies of the finer structures of the nervous system has been attained only recently. This circumstance explains the lack of adequate procedures for this investigative method.

In 1932 M. I. Gramenitsky [3] suggested microscopic observations on a living preparation of a distended frog heart using a penetrating light. Soon, from the laboratories of D. N. Nasonov and B. I. Lavrentyev there appeared a series of studies on the morphology of nerve cells (in the excitatory or phase of injury states) the material having been taken from frog intramural cardiac ganglia. Applying such stimulators as chemical, mechanical and electrical ones to the Gramenitsky preparation D. N. Nasonov, B. I. Lavrentyev and their pupils were able to follow experimentally the changes in the aggregate state of the neuroplasm, the nucleolus and the nucleus, as well as the pericellular apparatus.

In the later years, the penetrating-light method of vital microscopy has attained an increasing development. The technique has been developed by P. A. Fonviller [5], A. A. Vishnevsky and B. I. Lavrentyev [1, 2] and their students. This procedure differs from the Gramenitsky preparation in that it allows the study of the finer nerve structures while the circulation is intact, and also permits study of opaque or only slightly translucent organs so that the two methods can be used very satisfactorily to supplement each other.

Unfortunately, the penetrating-light method is effective for nerve studies only after a preliminary staining with a weak methylene blue solution, which acts as a stimulator. For this reason the Gramenitsky preparation is to be preferred, as it permits study of such fine structures as the pericellular apparatus without stains.

In developing the technique of vital microscopy for the study of the nervous system, we searched for a method which would combine the virutes of the two described methods. We believe we were successful in making such a preparation from the urinary bladder of the grass frog, which has been long used in vital microscopy of the circulation.

The preparation so made enabled us to make observations in penetrating light at all microscopic magnifications and with the preservation of the blood circulation, nervous connections and nerve fibers since the operated frog (with good care) can live for months.

To make these preparations, we took mainly larger grass frogs, male and female, weighing no less than 50 g, since the larger the animal, the less the necessity of resorting to a magnifying glass to control the course of the operation.

To immobilize the frogs, we used native curare-like diplastin* allowing 0.02 mg per g weight of animal. Such a dose weakens the skeletal musculature without impairing the respiration, over a period of some 4 hours.

* Russian trade name.

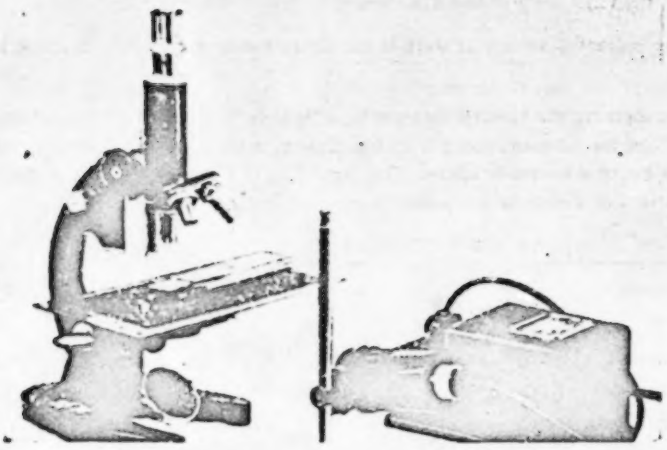

Fig. 1. General view of the special stage replacing the usual objective stage of the microscope.

If the experiment continued for a longer period, an additional half-dose injection would be given. Diplastin can be given intramuscularly or injected into the lymphatic sac.

Ringer solution was used for moistening the wound and skin surfaces.

The procedure consists of three stages: 1) preparation of the urinary bladder, 2) placement of a special stage on the microscope, 3) fastening the body of the frog and its urinary bladder to the special stage.

1. Preparation of the urinary bladder.

Essentially, this stage consists in extirpation of the wing of the iliac bone, free dissection of the corresponding flap of the urinary bladder and bringing it, along with the colon, outside the abdominal cavity. It is easier to work from the right, as the colon lies mostly on that side in the frog.

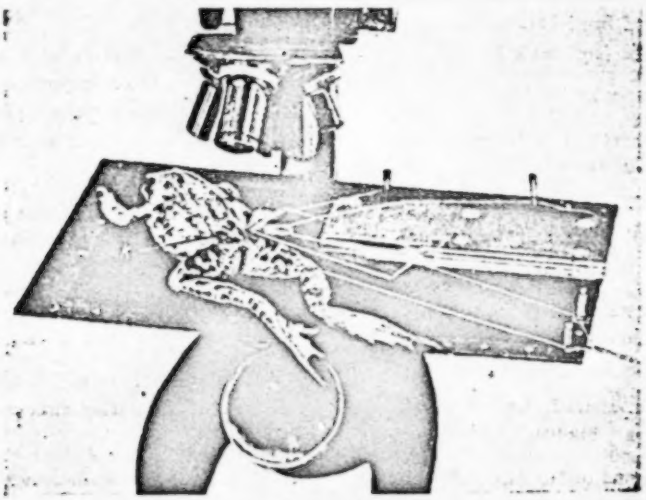

Fig. 2. General view of the complete preparation along with the needle used for intra-arterial infusions. k) Needle cannula.

Hemorrhage is avoided by the ligation of the spinolumbar vein and then the femoral artery before undertaking the extirpation of the right wing of the iliac bone.

The ligation of the indicated vessels as well as the dissection of the bladder is aided by the use of a magnifying glass.

Immediately after severing the bladder ligaments, a ligature is placed at the junction point of the large and small intestines. Then the bladder, along with the cloaca, is brought to the outside and filled with Ringer solution so that ligatures become easier to apply. The right flap of the urinary bladder then acquires a spherical shape while the spinal artery of the urinary bladder moves to a middle position.

2. Placement on the microscope of a special stage.

The ordinary microscopes M-9 and MBI-1 were used. We prefer the MBI-1 as the angle of its tube considerably simplifies the task of the investigator.

We replaced the ordinary objective stage, along with both its regulatory screws, with our special stage. The general view is as seen in Fig. 1. This stage consists of a right-angled board of about 2-3 mm thickness having small openings at the edges through which ligatures can be passed. In the center of the board is a round opening whose diameter must be no less than 4.5 cm. At the side of the aperture in the board there is fastened an immovable block having a groove for the objective slide (76 × 26 mm). This last is placed parallel to the board of the special table and is separated from it by a distance of 8 mm.

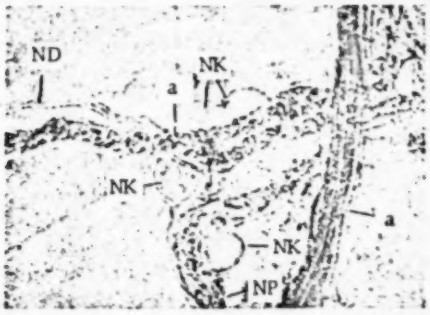

Fig. 3. Small ganglion in the nerve plexus of the urinary bladder of the grass frog. Film frame; obj. 20, ocular 10. a) Arteriole; np) nerve bundle; nk) nerve cell.

After the stage has been placed, lighting is arranged by the Köhler method [6].

3. Fastening the body of the frog and its urinary bladder to the special stage.

To fasten the body of the frog and to stretch the urinary bladder, no less than 4 ligatures must be placed: one each on the large bowel and the uper portion of the right shin, and two for the urinary bladder. The latter two are placed symmetrically, one on each side of the spinal artery, somewhat below the point where it branches into small arterioles.

The ligatures are placed by means of fine intestinal needles. After this is done, the Ringer solution is poured away from the bladder region.

The ligatures from the right shin and the colon of the frog are drawn to the objective glass so that the latter will rest on the cloaca. It should be borne in mind that inadequate fixation of the frog body during the experimental period inevitably leads to overstretching of the straightened urinary bladder and this, therefore, will disturb the circulation.

After careful placement of the ligatures fixing the body position of the animal, the bladder is straightened out, keeping in mind at all times that drying the urinary bladder surface will also diminish the flow of the blood in the vessels.

Chemical solutions may be introduced into the blood stream by means of a syringe attached to the No. 14 needle which has been placed into the lumen of one of the sciatic arteries. Figure 2 presents a general view of the preparation, the needle being in place.

As the preparation is viewed, the first thing observed is the blood circulating through the numerous vessels and capillaries of the urinary bladder.

Against the background of this circulation no staining is required as long as there is enough illumination that we may observe with sufficient clarity the intramural nerve plexus which consists of three gnaglia connected with each other by powerful nerve strands. This plexus lies on the dorsal wall of the urinary bladder between

the neck and the center of the flap being investigated. The ganglia vary in size: the smallest may contain only 6 nerve cells (Fig. 3), the average have 10-11, while the largest may have around 20 cells. The ganglia of the urinary bladder are united by means of fairly thick nerve strands with the sciatic-caudal and intramural plexi of the cloaca and rectum. In the other directions from these same ganglia, numerous strands of nerves entwine the entire urinary bladder.

The very finest nerve structures which we have uncovered by the use of this method we shall detail in subsequent communications.

SUMMARY

A special method for the in vivo study of the frog urinary bladder nerves is presented with illustrations. Subsequent communications will present additional material.

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GRAPHIC RECORDING OF POINT PERISTALSIS IN STOMACHS OF DOGS

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(Received October 14, 1956. Presented by Active Member Academy of Medical Sciences, Prof. V. V. Parin)

There are numerous methods available for recording gastric motility [1, 2, 3, 4, 5]. However, the gastrogram recorded by means of these methods is the result of many factors: tonic gastric contractions, its peristalsis, unevenly timed and varying in strength and rhythm movements in different portions of the stomach, alterations in the pressures within the stomach produced by respiration, animal movements, even, for example, turning the head and moving the legs, etc. The character of the gastrogram fluctuates continuously depending on which movements are affecting it at any given moment, and to what degree.

As we studied gastric motility under various conditions affecting the organism, we came to the conclusion that it was essential to differentiate gastric peristaltic movements from other forms of its movements in order to be able to follow the pure dynamics of peristalsis itself. This can be attained by graphic registration of so-called point peristalsis, i. e., peristaltic movements originating in one small gastric area.

EXPERIMENTAL METHODS

The method we propose consists of the following. Through a fistula tube in the stomach of a dog, there is introduced a thin-walled balloon (usual children's toy sphere) with a capsule glued to its outside (2) which absorbs the peristaltic wave (Fig. 1). The balloon (1) communicates by means of a wide rubber tube (3) with a water manometer which assures the extended placement of the balloon and the pressure within it equaling that within the stomach.

The capsule (2) is made from the finger of a surgical glove and a rubber stopper (from a penicillin flask). This latter is of a thickness 1.5-2 mm and is glued to the base of the capsule (2) and serves to assure pressure stability within the capsule during changes in pressure occurring within the balloon. With the aid of a nipple tube (5) which, while within the region of the balloon and the stopper of the fistula, passes within the rubber tube uniting the balloon with the manometer, allowing the capsule (2) communication with the Marey capsule. Preparatory to registration, it is necessary to insert the balloon into the stomach, fill balloon and capsule with air, and then place the balloon in such a manner that the highest waves would become recorded.

The balloon and capsule are filled with air through stopcocks (6) and (7). The balloon is inflated to a volume of 200-300 cc, as is the capsule (2), until the membrane is observed to be obviously stretched. The Marey capsule is of less than average size; its membrane is made of the thinnest and most elastic rubber available.

The balloon is placed by rotating slowly the fistula tube until the recording device records maximal fluctuations. Occasionally, the fistula tube has to be fixed at the place found to be most advantageous for the balloon. This fixation is readily accomplished by the aid of a roller bandage pulling the fistula tube against the rubber tube (3 or 10) as indicated.

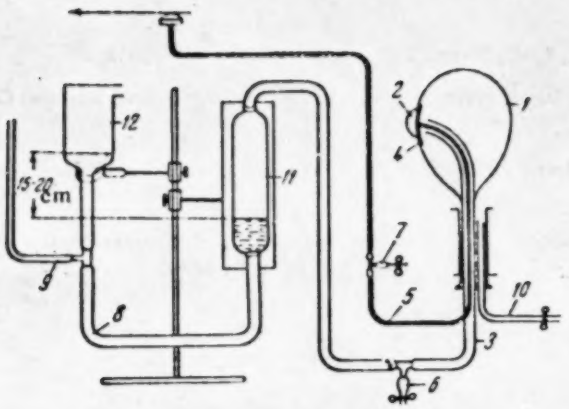

Fig. 1. Scheme of the installation for graphic registration of dog point gastric peristalsis. 1) Balloon absorbing all changes in pressures within the stomach and transmitting them to the manometer; 2) capsule absorbing the perstalsis in a small area of the stomach; 3) rubber tube connecting balloon with manometer; 4) thick base of capsule assuring pressure stability within it as pressures fluctuate within the balloon; 5) nipple tube connecting capsule to recording device; 6) stopcock for filling balloon with air; 7) stopcock for filling capsule with air; 8) rubber manometer tube; 9) side tube on Marey capsule for recording the general gastrogram; 10) rubber tube for introducing food into stomach; 11 and 12) wide flasks serving as manometers.

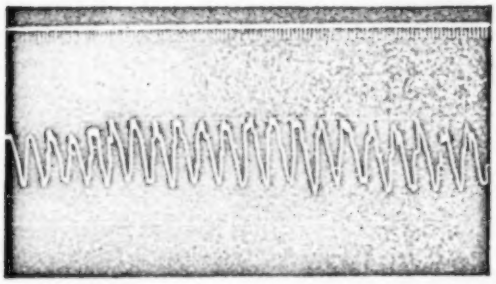

Fig. 2. Recording of a peristaltic wave.

After the balloon and capsule (2) have been filled with air, we have two closed systems: balloon–manometer and capsule (2)–Marey capsule.

The influence of pressure alterations within the stomach is either fully nullified or markedly weakened by the balloon–manometer system and so is not reflected in the capsule (2)–Marey capsule system. The thick foundation of capsule (2) hampers this. When, however, a peristaltic wave passes over capsule (2), a maximal effect is obtained, as its pressure does not have to overcome the muffling effect of the thick base (4) lying on the balloon (1). The peristaltic wave is then readily recorded as shown on Fig. 2.

EXPERIMENTAL RESULTS

We advance our method of graphic registration of point peristalsis as a very useful maneuver. The method does not demand much time for its setup and, most important, permits the differentiation of peristaltic waves from other stomach movements. When two instead of one absorbing capsules are placed on the balloon at a known distance from each other, it is possible to determine the velocity of the peristaltic wave. The setup can be used for obtaining simultaneously the usual gastrogram. To do this, it is necessary only to connect tube (9) by a side-arm leading to another Marey capsule.

SUMMARY

This method is offered as a procedure for recording the peristaltic movements of the stomach and eliminating the extraneous influences of other movements. It is simple and allows precise localization of the waves and also the recording of the peristaltic velocities.

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* In Russian.

A METHOD OF PRODUCING EXPERIMENTAL SCALDS

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(Received November 1, 1956. Presented by Active Member Academy of Medical Sciences, Prof. V. N. Chernigovsky)

Our problem consisted in devising such an experimental model of burn production that we could trace the course of the disease from the initial burn shock through to the final healing of the wound produced. There was no effort made to devise therapeutic procedures to be used on the experimental animals.

Most therapeutic investigators have approached individual facets of burn problems such as treatment of burn shock, acute toxemia or local burn therapy, and so have devised production of burns to suit their individual needs.

The majority of authors studying experimental burn shock produced burns either by scalding or with flame [1-9]. As can be seen from the reading of their work, they produced burn shock in animals by causing second- and third-degree burns over 30-60% of the body areas.

Even under such conditions, shock was not always evoked, as we convinced ourselves when we employed as the burn agent a combustible mixture having a high temperature of flaming. Second- and third-degree burns covering 30% of the body area would not lead to the development of burn shock.

It is evident from this discussion that before an investigation of therapy for burn shock can be undertaken, it is essential to develop a reliable method whereby burn shock can be evoked consistently.

As is well known, the following conditions are a prerequisite for the production of shock: a definite area of involvement, intensity and duration of action by the exciting agent.

Taking all these factors into account we set ourselves the task of finding such a method of producing burns which would lead to shock when tissues of a relatively small area would be involved to not an excessive depth, and when the animal could be observed through the healing stages after it had been led out of shock.

After investigating such shock-producing burn methods as scalding water, flame and superheated steam, we came to the conclusion that when a small area involving tissues rather superficially and yet reliably leading to shock is to be used, it is necessary to decrease the temperature of the burn factor while increasing the duration of its action.

In our experiments, these conditions were met by a method which used superheated steam as the agent inflicting the burn. A similar method for producing burns involving not over 10% of the body area was evolved by V. I. Muraviev and A. K. Kozyrko in 1952 when they employed steam superheated to 200°.

EXPERIMENTAL METHODS

Steam, leaving the usual medical autoclave at 2 atmos of pressure, enters a rubber flange 2,5 meters in length attached to the outlet valve of the autoclave. To the free end of this flange is attached a spirally coiled copper tube, 0.8 cm in cross-section, which is placed over the tube of a soldering lamp (Fig. 1). This blow torch produces a flame which incandesces the copper coil and superheats the steam within it. This superheated steam exits at 200°, the temperature diminishing as the distance from the free end of the coil increases (Fig. 2).

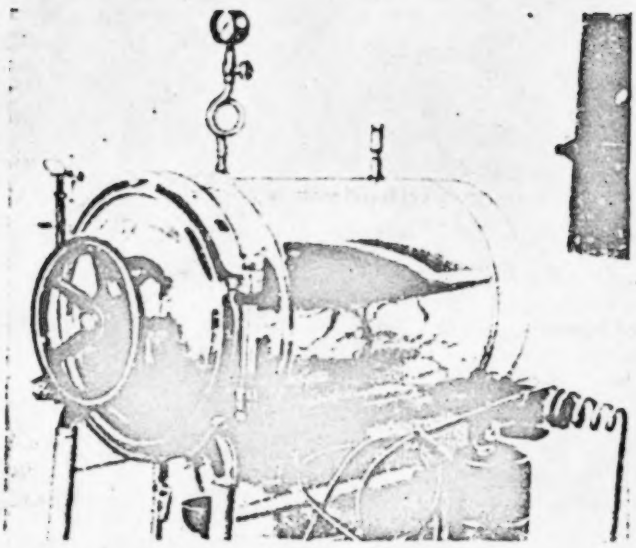

Fig. 1. Autoclave with the attached rubber flange which is placed over the spirally coiled copper tubing fastened to the tube of a soldering lamp.

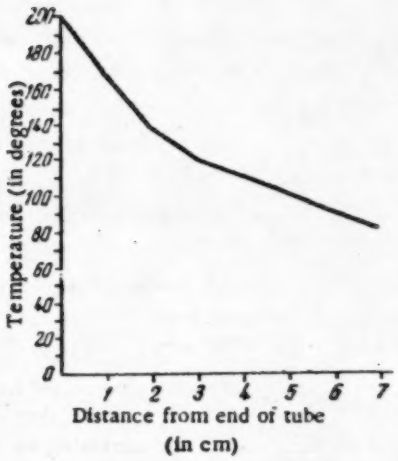

Fig. 2. Temperature changes of the superheated steam at various distances from its point of exit from the tube.

In order to inflict the burn, we fastened the dog lying on its back. The skin, over an area embracing the lower sides of the chest, abdomen and inner surface of the thigh, was shaven to embrace a skin area approximating some 15-17% of the total body area. The cardiac area and the right groin (where the arterial femoral cannula for the recording of arterial blood pressures was placed) were protected from the burn by tampons clamped to the skin.

To produce the burn, the blow torch, along with the tube attached to it, was brought to the shaven area so that the free end of the tube was 3-5 cm away from it. At such a distance, the steam has a temperature of 100-120° (see Fig. 2).

Under such physical conditions, the end of the tube was passed evenly over the entire shaven area, a burn being inflicted for a period of 12-15 minutes.

There would result on a sharply demarcated area a 2nd- to 3rd-degree burn while, at the same time, the protected unshaven area would not be involved.

Such a method permits observation of the animal from the moment of infliction of the trauma, and the basic clinical tests can be recorded.

The burns produced by superheated steam produced severe shock in 70% of cases as characterized by the following clinical tests: decrease of arterial pressure as compared with initial readings by 30% and more, tachycardia with a feeble pulse, shallow and labored respirations, weakening and up to disappearance of such unconditioned reflexes as response to pain, pupillary, corneal and blinking stimuli, fall in body temperature and in-

creased thickening of the blood. All the experimental animals which died did so either in the first hours following the burn, or, in some instances, at the end of the first day or beginning of the second. In the other 30% of instances, the dogs developed burn shock of much lesser severity, as manifested clinically by the signs just described. All these animals emerged from shock by themselves. Later, they could be followed through the successive stages leading to complete recovery.

SUMMARY

A simple method is described for producing burn shock with the use of superheated steam. When 15-17% of the dog's body is exposed to this steam at a temperature of about 120° for about 15 minutes, 2nd and 3rd burns will lead to fatal burn shock in about 70% of the cases, the other 30% recovering spontaneously and going on to healing. This method is offered, then, as being useful for future studies to be undertaken in the therapy of burns.

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A METHOD OF PREPARING HEMOGLOBIN – FREE SERUM

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(Received November 16, 1956. Presented by Active Member Academy of Medical Sciences, V. N. Chernigovsky)

Existing methods for obtaining blood sera depend on the fact that whole blood coagulates in vitro. As a rule, these methods permit some admixture of hemoglobin in the serum. In cases where this slight admixture of hemoglobin has no practical significance, these methods are adequate.

However, in tests such as photometric analysis and setting up colloidal reactions, for example, the presence of even a small amount of hemoglobin in the serum can lead to serious errors. In animals such as dogs, the erythrocytes are fragile and can break up during manipulations needed to obtain the serum. To obtain hemoglobin-free serum from them is no easy matter.

We decided to look for a method for obtaining blood serum free of hemoglobin. We developed a method involving the clotting of plasma instead of whole blood, as done in other procedures.

Paraffined centrifuge tubes could be prepared on the day (or day before) the blood was collected. Carefully washed and dried tubes were taken and filled with melted paraffin which would be immediately poured out, the tubes being then cooled. The walls of the tubes would thus be covered with a thin paraffin coating. Some 20-30 minutes before drawing the blood, we placed tubes in an ice pack, thus assuring their refrigeration.

In addition to the paraffined tubes, an equal number of well-dried centrifuge tubes is needed.

To draw off the plasma, there should be on hand several pipettes having rubber pyriform attachments.

The blood must be drawn from the dogs by means of short needles having large openings, but thin walls. One may employ needles used for spinal punctures, but these should be shortened to a length of 3-3.5 cm.

The anterior external metatarsal vein of the dog is pierced without application of a tourniquet, or by only applying one at the very moment of entering the vein with the needle. The blood flowing freely through the needle lumen is collected into the refrigerated tube. The effort should be made to have the blood drop directly to the bottom without flowing down the sides.

The blood is immediately centrifuged at 2500 rpm for 3.5-4 minutes. In this time interval, the formed elements of the blood are fully precipitated, while the plasma has had no opportunity to clot.

The blood plasma is carefully taken up by the pipette having the rubber bulb enlargement on it and transferred to the dry centrifuge tube.

To hasten coagulation of the plasma in the tube, it is placed in a thermostat at a temperature of 37-38° for 30-40 minutes.

The coagulated plasma is then placed in a refrigerator at 3-5°. Here clot retraction occurs.

After 20-24 hours, the tube is taken out and a fine glass stick is used to free the clot from the centrifuge tube walls after which it is centrifuged at 2500 rpm for 2-3 minutes. As a result, the plasma clot, consisting

of fibrin threads, contracts and can be found at the bottom of the tube under the layer of the serum. This hemoglobin-free serum is then poured into a clean test tube.

From 10 cc of blood there is obtained 3-4 cc of serum.

The most important technical factor in the entire procedure of obtaining hemoglobin-free serum lies in the necessity of having all formed elements precipitated before the blood is permitted to clot. For the adequate fulfillment of this requirement, the procedure as just outlined has to be adhered to rigorously.

SUMMARY

A method is outlined whereby hemoglobin-free serum may be drawn from dogs. It depends on the precipitation of the formed elements before clotting has taken place.

MEASUREMENT OF ARTERIAL PRESSURES IN INTACT RABBITS

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The most popular methods of taking indirect arterial blood pressures in rabbits under conditions of a long-range experiment utilize the central ear artery [3] or else an external skin flap holding the carotid artery [2]. Many fairly successful modifications of these procedures do not remove their serious defects.

In the first place, there is no way of recording the very high sensitivity of the rabbit ear vessel network, as shown by rapid and considerable blood stream fluctuations in response to the most varied exogenic and endogenic factors. Actually, the very act of taking the blood pressure markedly affects the width of the vessel lumina. The use of this method permits only a moderate accuracy in measuring the systolic pressure alone.

In regard to taking carotid arterial pressures, there is the obvious previous surgical interference which is not altogether physiological. It is well known that interference with carotid flow and mechanical irritation of the arterial stem leads to general reflex alterations in the circulation. The disadvantages of these methods become still more evident when repeated readings are taken over a short time interval, especially in animals already having a disturbance of the regulation of the cardiovascular system.

Having measured blood pressures in intact dogs by the Korotkov method, and being convinced of its simplicity and reliability, we applied this method in rabbits. In spite of the almost universal application of the Korotkov method in medicine, it is used relatively little in the physiological practice of taking pressures in animals. This is in spite of the fact that auscultatory methods have the very important advantage of determining both the systolic and diastolic pressures quickly and accurately.

In view of the lability of rabbit blood pressure, one should avoid fixation methods exciting the creature. Thus, for example, a rabbit tied in the usual manner to a table has a much higher pressure than does a relatively free animal. At the same time it is obviously necessary that the rabbit be more or less immobilized so that its movements would not affect the readings. For fixing the rabbit in one position, the stand described by A. M. Ivanitsky [1] has been found most useful. We altered its construction only slightly, cutting right angled openings 10 × 10 cm in the anterior part of the side walls; these openings could be closed by means of little wooden doors. These cut-outs are needed to permit free access to the fore limbs of the animal.

To take blood pressure in adult rabbits, we made a cuff measuring 2.7 × 10 cm, which is attached to the shoulder by means of metallic clips. The cuff fits snugly to the shoulder, but does not compress it. When the cuff is applied too loosely, the pressure readings usually rise; while a too-tightly-applied cuff soon leads to the appearance of stasis phenomena. A T-tube was used to connect the cuff with a glass jar holding 500 cc thus markedly increasing the volume of the system, and allowing gradual and smooth alterations in the pressure within the cuff.

The Korotkov sounds in a rabbit are clear and distinct, even if much feebler than in dogs. In rabbits, the phases of the sounds cannot be distinguished as they can in man and dog. With just a little practice, the tones

can be readily heard in the radial artery as it passes over the medial aspect of the elbow. The moment of appearance and disappearance of the sounds is best determined during gradual deflation of the cuff; usually, there is no difficulty in hearing them.

The blood pressure of adult rabbits, as measured by this method, varies within 95-120 mm Hg pressure for the diastolic reading and 110-140 mm Hg for the systolic pressure reading.

Occasionally, some rabbits exhibited asymmetrical blood pressure readings.

In comparison with dogs, the rabbit blood pressure readings exhibited much greater lability. In our opinion, this is associated not only with species differences due to rabbits having such sudden and energetic movements accompanied by corresponding rapid hemodynamic alterations, but also is associated with a less developed, less "economical" mechanism regulating blood pressures in animals lower on the scale of mammalian evolution.

SUMMARY

Measurement of arterial blood pressure in intact rabbits is described by Korotkov's method. Animals were fixed to a special stand during investigation. A pneumatic cuff, measuring 2.7 x 10 cm, was applied to the shoulder. Korotkov's sounds were auscultated with a phonendoscope on the radial artery from the medial surface of the elbow joint. Systolic pressure in adult rabbits ranged from 110 to 140 mm Hg, while the diastolic was from 95 to 120 mm Hg.

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July, 1957

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[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratorii	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologii	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologii	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologii, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimii	Uspekhi Biokhimii	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologii	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirurgii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologii	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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